ST. LUKE'S HOSPITAL

Medical and Surgical Reports

Volume IV

1917

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Medical and Surgical Reports

Volume IV

1917

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It has been customary at St. Luke's Hospital for a number of years past to collect some of the papers written by members of the attending staff and publish them in permanent form for convenience of reference, the issuance of these volumes having been made possible by a donation from a friend of Dr. Austin W. Hollis. Some of the papers in the present volume, Volume IV of the series, have been previously published in medical journals; others have not heretofore appeared in print. In response to repeated requests from internes and graduates of the hospital, the technical methods employed in the pathological laboratories have been published in this volume in extenso*. These directions do not include all the methods which it is possible to employ in a hospital laboratory, but are merely the general procedures which are in use in the St. Luke's laboratory and which have been heretofore circulated in typewritten form for the guidance of the internes in the laboratory; as such they represent merely the minimum requirements.

Committee on Report of the Medical Board,

Robert Abbe, M. D.
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* Reprints of the technical methods will be published for those desiring extra copies, and will be placed on sale by James T. Dougherty, 409 West 59th Street, New York.

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UTERINE FIBROIDS CURED BY RADIUM

ROBERT ABBE, M. D.

A new chapter in surgery must be written on the treatment of uterine fibroids. These are by far the most common of all pelvic tumors coming to the surgeon for relief; the majority do not cause inconvenience until hemorrhage calls attention to them: and they often grow to large size without causing annoyance and usually without inducing adhesions in the peritoneal cavity. When they cause menorrhagia it is due either to the stretching of the lining membrane of the uterine cavity over the rounded surface of the tumor bulging inward (on which one or more open mouths of small veins can be demonstrated after operative removal of the uterus), or to the spongy vascular change of the lining, which bleeds at menstrual congestion or when touched by the probe.

Surgeons have been universally prone to advise removal of the tumors, with uterus, ovaries, and tubes. This cures the patient undoubtedly, and in the past it has been the best treatment. It is no longer so; the cost in risk and time is too high. Radium can do better. In the hands of a good surgeon, the operation is a fairly safe one, but as performed by the average surgeon far and wide throughout the country there have been a discreditable number of deaths, a not uncommon sequel of pelvic abscess, of abdominal hernia, of abdominal wall infection abscess, of phlebitis, or, not the least unpleasant, of post-operative conditions, such as long convalescence with neurasthenia, or excessive increase in weight, following ovarian castration. In any event, the patient is disabled for from one to three months. Moreover, relief is often required in the face of grave anemia which adds to its risk and sometimes prevents operation entirely.

The simplified radium treatment now comes to the front as an unrivalled substitute. More than ten years' trial has demonstrated its great efficiency, so that in most cases it may supplant the operation for the following reasons:

1. It is harmless and painless.
2. It does not interrupt the patient's work.
3. It stops hemorrhage.
4. It brings about a slow absorption and disappearance of the tumors.
5. The patient is saved the mutilating operation and the sequelæ of ovarian castration.
6. Up to the present experience it seems to be uniformly efficient in all cases of fibroid tumors of the uterine wall, no matter how large.

As far as is known, my own application of radium for this purpose, in 1905, was the first on record. I based my use of it in the uterus on my successful work in blocking all vascular tumors and nævi by radium after the recommendation and practice of Wickham of Paris. A tube of strong radium applied to the bleeding lining of the uterus for one or two hours will surely bring about a change in all veins and arteries near it, which will partly, or wholly, close many of them and end the hemorrhage. This endarterial change should be allowed six or eight weeks' time to induce the correct reduction or produce obliteration. After that time, if bleeding continues, a second application may be given. My experience with this method convinces me that it is often permanently effective after two such applications. Nevertheless, for various reasons I have given several applications at long intervals when I was feeling my way to find an efficient and safe dose in the early days of its use. At the present time my belief is that one initial treatment of two hours' duration is correct, to be repeated in two months if hemorrhage continues.

By this means an alteration of the diseased uterine lining is accomplished without risk of sloughing or cicatrization. What is accomplished is a partial occlusion of the exaggerated vascularity, with subsequent mild fibrosis or atrophy, the aim being to keep a good mucous lining. This is accomplished by the

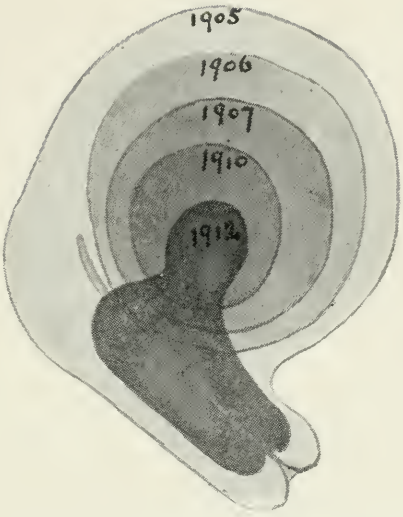

Fig. 1.—Case 1, Progressive reduction of fibroid tumor after the use of radium in the uterus; side view.

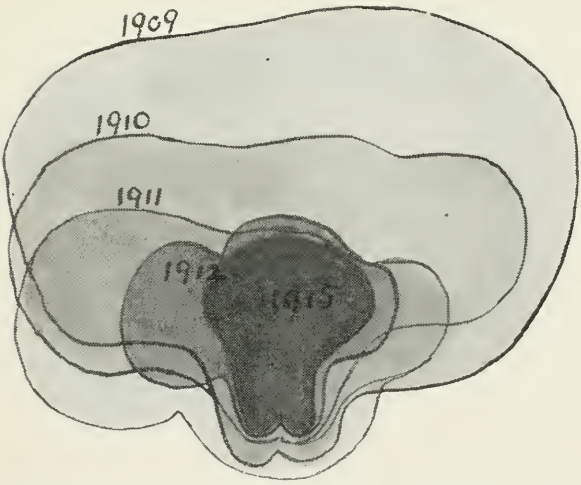

Fig. 2.—Case II, Progressive reduction of fibroid tumors after exposure for four hours to 35 mgm. radium element in the uterus; front view.

efficient beta radiation from the tube, as applied by my method, which activates the tissue to a depth of an eighth of an inch at least, which is desirable in tissues causing menorrhagia. At the same time the enormous output of gamma radiation penetrates all the uterine tumor and other structures to a depth of one or two feet, and, by generating beta rays throughout the resisting myomatous tumor cells (as demonstrated by experimental proof[1]), brings about a retrograde metamorphosis which begins promptly, but is usually not measurable for a few months. The subsequent shrinkage progresses indefinitely and the tumor ultimately disappears. There are probably some variations in tumors, but all seem to yield.

The accompanying two illustrations, Fig. 1 and 2, of my earliest cases are incontestable evidence of its efficiency and persistence. These were shown at the exhibition of the International Surgical Society in London and at the British Medical Association in 1913, at Brighton, England, and prior to that at the New York State Medical Society meeting at Rochester.

In writing of this important subject previously[2], I reported the case of a very large fibroid in an elderly lady who was exhausted by profuse and frequent hemorrhages and in whose case it was not wise to consider operation. Two radium treatments were given a few days apart with no pain or inconvenience to the patient. After this, two periods gave a slight extra flow, but the patient gained in color, health, and strength, and has not ceased to remain perfectly restored to health and color, with no return of menorrhagia. The tumor has shrunk little by little till now after nearly a year it is one-sixth its former bulk. The illustrations show beautifully the through and through radium radiation from the uterine cavity to the abdominal wall, through a twelve inch mass. The reduction is shown in the two outlines in Fig. 3.

Since reporting my first twelve cases in private work[2], I have treated thirteen additional ones.

My conviction is fully confirmed that surgical science has few more striking triumphs to its credit. This method not only makes the surgeon the true helper, but saves the patient from the terrors, the risks, and the sacrifices of surgery.

Technique: Preliminary preparation seems unnecessary. The diagnosis having been made and precise notes recorded, the cervix is tightly held by a single hook tenaculum and freely painted with iodine. A small swab saturated with iodine is then carried into the uterine cavity. The canal is usually found fairly open, though occasionally in virgins it requires gentle dilatation. The

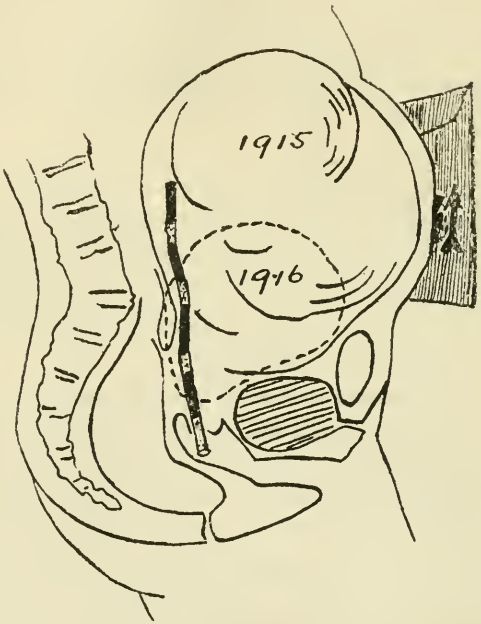

Fig. 3. Effect of radium radiation through twelve inches of tumor and abdominal wall on a photographic plate with lead devices placed externally. The dotted line represents the reduction of the tumor after one year. Two applications of 75 milligrams radium element, for two hours each, wholly stopped the exhausting hemorrhages and restored the patient to health.

presence of intrauterine tumors usually induces automatic dilatation, as if in anticipation of extrusion. The uterine sound now takes the measure of the cavity and records any internal distortion. The radium applicator, to be described, is gently pressed into the uterus, as far as it will go, and a sterile gauze bandage packing placed around it as the speculum is withdrawn. The patient may now step off the table and lie for two hours on a sofa,

Fig. 4

Fig. 4.—Radiogram after 2 hours' exposure to 100 mg. radium element in utero, taken through 12 inches of uterine tumor and abdominal wall.

after which the tube and packing are withdrawn with no further precaution, and the patient may be allowed to go about as if nothing had been done.

There is no unpleasant sequel to this as far as my experience goes, either in temperature or pain. The activation of the tissues due to the radium application begins, of course, at once, but is not noticed by the patient. It corresponds with that which we see on the skin, a sharp radium reaction showing in ten days, continuing ten days, and subsiding during the third ten days. In the uterine tissues, the sequel is a vascular blockade of at least a portion of the overgrown vessels of the endometrium, which results in arresting hemorrhage in the ensuing weeks, while the shrinkage of the tumor due to retrograde change in the myoma cells follows and continues for months and even years without further treatment.

The method of using radium in this field is by having it sealed in a thin glass or platinum tube of the diameter of a match stick, and not over an inch long. Three such tubes, each holding approximately twenty-five milligrams of the radium element, will suffice for the treatment of any hemorrhagic fibroid. These are placed within a larger tube for safety. After much experimenting with quills, vulcanite, gold, silver, and aluminum, I have found that the best substance is a celluloid tube, one-quarter inch in diameter and four inches long. This is about the thickness of a goose quill and while easily slipped into the uterus offers no resistance, to the effective passage of the beta or gamma rays. No metal filter is interposed between the radium and the uterine lining, so that the efficiency of the beta rays is obtained. (Fig. 4.)

The small radium tubes are placed within the celluloid holder, separated by a bit of cotton, and the open end is closed by wrapping the end of a strip of adhesive round it, pinched together along its length, as a convenient handle for removal after use. The holder is put into a bottle of iodine and left during the preliminaries, (Fig. 5) till it is taken out, dripping with iodine, and pressed into the uterus.

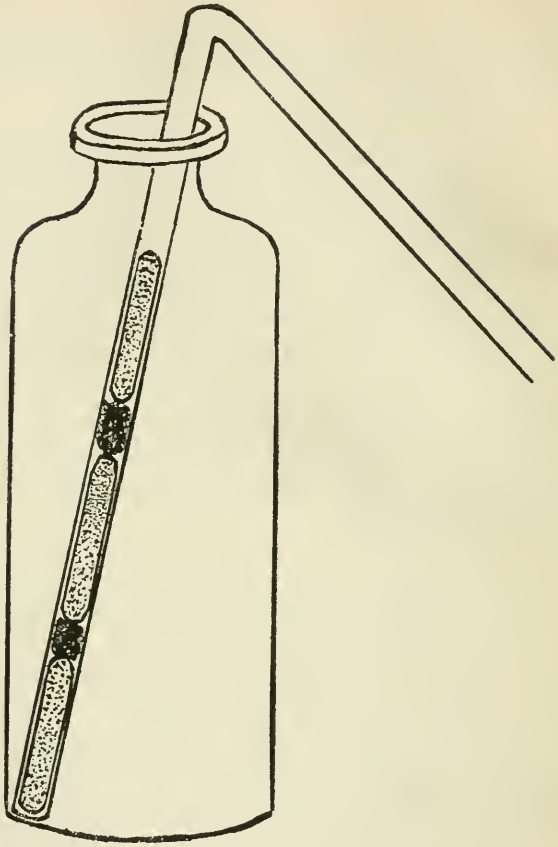

Fig. 5.

1. Abbe. Med. Rec., 1914, lxxxvi, 909.
2. Abbe. Med. Rec., 1915, lxxxvii, 379.

POISONING BY MERCURIC CHLORID AND ITS TREATMENT*

SAMUEL W. LAMBERT, M.D., AND HENRY S. PATTERSON, M.D.

The recent increase in the number of cases of poisoning by mercuric chlorid is largely due to the newspaper notoriety which was given to a case of accidental poisoning occurring about four years ago. The public were instructed in all the details of the symptoms, and great stress was laid, not only on the sureness of the fatal ending, but also on the painlessness and lack of suffering which accompanied the week-long illness of that particular victim. The repetition of similar publications and the ease with which tablets of the drug could be obtained appealed to would-be suicides, and now both intentional and accidental cases of poisoning have become a common occurrence in hospital practice. Mercuric chlorid tablets have been used for some years as a household article to treat the ordinary slight wound infections of the family, to rid the house of bed vermin, and in many cases as a preventive of conception.

The commonest accident resulting in poisoning is the swallowing of the tablets in mistake for some headache remedy: such accidental cases about equal in number those of attempted suicide. In New York City the cases of suicide by poisoning, due to the principal drugs, for the past twenty-five years, are shown in Table 1, arranged in five-year periods.

This table shows that there is a fashion in the methods of suicides, and that poisoning by arsenic and Paris green and by phenol (carbolic acid) are on the decrease, that by opium is stationary, while poisoning by illuminating gas and mercuric chlorid are both becoming popular methods and are on the increase. Of the 155 cases of suicide by mercuric chlorid recorded in the past twenty-five years, seventy-three, or nearly half of

* Reprinted from *The Archives of Internal Medicine*, November, 1915.

TABLE 1.—Suicides in New York by Poisoning, 1890-1914

Poisons	1890-1894	1895-1899	1900-1904	1905-1909	1910-1914
Paris green and arsenic	155	170	99	47	48
Phenol	73	627	1,455	325	154
Opium and morphine	42	56	56	48	29
Mercuric chlorid	4	10	9	28	104
Illuminating gas	65	315	628	1,149	1,554
Other poisons	58	94	104	196	237
Total all poisons	397	1,272	2,351	1,793	2,126

Figures from 1890 to 1897 inclusive represent former City of New York.
Figures from 1898 to 1914 inclusive represent Greater New York.

them, have occurred in the last two years. This question, therefore, forms an important social problem. The regulation of the sale of the poison in the form of antiseptic tablets of corrosive sublimate is already begun, and should be undertaken and enforced by all boards of health.

The symptoms of poisoning by mercuric chlorid came into prominence and were studied first as a complication of surgical operations and in obstetric practice. The drug was introduced as an antiseptic in the early eighties, and has been widely used ever since. In the cases of wound absorption, the initial toxic gastritis is absent, and the kidney symptoms and salivation are less prominent than the colitis. The absorption of the drug is gradual in these cases, and its effect is shown in great part in the efforts of the large intestine to eliminate it, with the result of an intensive and often fatal ulcerative colitis. A case of mercuric chlorid poisoning, resulting from the surgical accident of delivering about 1½ grains of the salt into the vein of a patient during a postoperative saline infusion, gave the following sequence of events, and throws much light on the symptomatology of the condition. The symptoms in this case are not the same as those which result from absorption from surgical wounds, but resemble the cases of poisoning with a predominance of kidney and intestinal symptoms.

The infusion was given on the fifth day after a laparotomy. Prior to the giving of the drug, the patient had passed about 30 ounces of urine a day,

and had had a daily evacuation of the bowels as the result of enema or saline catharsis. There was no previous vomiting, and the pulse ranged from 80 to 90. The wound was slightly infected, and the temperature ranged from 100 to 101.8. The infusion was given because of postoperative sepsis of mild type. Eight hours after the infusion, the patient vomited, and the pulse became irregular and weak. The vomiting was repeated several times during the first twenty-four hours. The secretion of urine was interfered with from the start, and about 2 ounces were secreted during this period. During the second twenty-four hours, 2½ drams of urine were obtained by catheter, and the bowels began to move involuntarily and continuously. The pulse was irregular and weak, the rate about 120 and the breathing was labored. The involuntary stools continued, and became bloody and full of mucus. Nine drams of urine were drawn by catheter during the day.

The following thirty-six hours presented the same symptoms of a severe colitis, and an almost total suppression of urine, and the patient died in extreme exhaustion five and one-half days after the infusion, without improvement in the colitis or the urinary symptoms. There was marked mental distress, but none of the ordinary symptoms of uremia.

A necropsy was held in this case, and its true etiology was first discovered by the microscopic appearance of the kidneys. The essential findings of the postmortem examination were as follows:

The heart was normal. The lungs showed some congestion and edema of lower lobes. There were large masses of clotted blood in the gastrohepatic ligament, the gastrocolic and the gastrosplenic ligament, and in the lesser peritoneal cavity. The gallbladder had been removed at the laparotomy. The liver was very friable and deeply bile stained. The bile ducts were much thickened and dilated, and contained many small black calculi. In the common duct there was a stone about 6 mm. in diameter. The spleen showed nothing special. The kidneys were very large and deeply jaundiced; there were several cysts on the surface. The capsule stripped with some difficulty, leaving the surface roughish. On section, the kidneys were very edematous and showed evidence of acute degeneration; the color was pale yellow with small hemorrhagic spots. The mucous membrane of the stomach was very much swollen, very red and covered with a layer of thick mucus. The intestines were filled with blackish watery feces, mixed with a large amount of mucus. The descending portion of the colon and sigmoid were covered with membrane and deeply congested. The lesion was most severe in the sigmoid. The glands were normal.

The anatomic diagnosis was fixed as: hemorrhage into the lesser peritoneal sac and gastric ligaments, chronic diffuse nephritis with acute degeneration, general jaundice, biliary calculi in bile ducts.

The microscopic examination of the organs showed biliary pigmentation in the liver cells and bile capillaries, and also in the kidney cells. The kidneys showed an extensive parenchymatous degeneration and calcification of the walls of the convoluted tubules, with large casts which are still present in the tubules. There is a small amount of connective tissue between the tubules. The glomeruli show no important lesions. These changes in the kidney are those characteristic of poisoning by mercuric chlorid.

In the cases of poisoning caused by swallowing the drug, the symptoms begin promptly after it is taken. Abdominal distress and pain, at first in the epigastrium and later more general, are the primary symptoms. Nausea and vomiting of the stomach contents and later of mucus, which is more or less blood stained, accompany the pains. This gastritis is usually promptly treated

with egg albumin or milk and lavage, and yields quite readily to such treatment, so that the patient is comparatively comfortable after thirty-six hours' rest and abstinence from food. The mouth shows signs of a mercurial stomatitis in about twenty-four hours, but this rarely is so severe as usually accompanies salivation from calomel. If the poisoning is left to run its course uninfluenced, the patient's attention is called to the diminishing amounts of urine in from forty-eight to seventy-two hours after taking the drug. This may develop to a complete anuria, and the patient dies without restoration of the secreting power of the kidneys. When this occurs, the patient may remain free from cerebral symptoms for the better part of a week, until a terminal form of low delirium supervenes and a weakening heart action proves fatal. There are none of the regular evidences of uremia, and convulsive seizures do not occur. If the kidney condition is survived and its function reestablished, and in those cases which do not develop this extreme degree of nephritis, there is apt to be a membranous colitis with deep ulcerations and hemorrhages, which may develop and prove fatal even after several weeks have elapsed from the time of the original poisoning.

In a case recently observed by one of us in consultation outside the hospital, a patient, who denied having taken the drug, had been anuric for three days. Calculus anuria was suspected, but an examination by Roentgen ray showed no stone, and examination of a stool revealed the presence of mercury. Free colonic irrigations were begun, and after five days of anuria, the patient passed 1 dram, on the following day 5 drams, and then the daily amount slowly rose until, after about a week or ten days of reestablished urination, he was passing over 50 ounces of urine. Notwithstanding this, at about the end of three weeks, he developed a severe colitis, with the passage of large casts of the colon, and died, after a large intestinal hemorrhage.

The therapeutics of mercuric chlorid poisoning up to the present have been quite unsatisfactory. The patients have regularly developed anuria on or about the fourth day after taking the poison, and progress to subsequent death, from lesions of the liver and colon, with or without the reestablishment of the urinary secretion.

Radical procedures have been tried in such cases, including the

surgical manipulations of loosening the kidney capsule; but it may well be doubted if such operative treatment has had any specific influence on the course of the clinical picture. The following treatment has been gradually formulated as the result of caring for ten consecutive cases of varying severity which have all resulted in a cure. The first two cases of poisoning resulted from the insertion of two tablets (15 grains) into the vagina, and was treated by favoring the elimination of the poison by frequent and prolonged colonic irrigations with normal saline and by such local measures as were needed to allay the severe vaginitis.

The remaining cases were due to swallowing one or more of the commonly used tablets containing 7½ grains of mercuric chlorid. The treatment in the detail now used at St. Luke's Hospital as a routine has been elaborated through the help of the method of Vogel,* by which mercury is readily detected in stomach contents, urine and stools. By this procedure one can determine not only the diagnosis and the necessity of beginning treatment, but also, in some cases, the length of time treatment should be continued. A certain number of patients are brought to institutions either pretending to have taken mercuric chlorid, or fearing that they have taken it by mistake in the dark, etc. It is particularly important to know if a patient has not taken the poison, for the treatment is most irksome to the patient, and entails unusual activity on the part of the nursing department.

Vogel's method consists of separating the mercury from its albuminous combinations in the organic material to be examined and then subliming it in a sealed tube to form an amalgam on a small piece of dentist's gold. He states that:

> If mercury is present it will manifest itself by the appearance of a silvery patch of amalgam on the gold foil. If the amount of the metal is exceedingly minute there will be simply a pale discoloration of the gold, seen to better advantage with the hand-lens or by removing the pellet from the tube and examining it with the low power of the microscope, but if the amount is larger the deposit on the gold will be very easy to recognize.

Before outlining the treatment, attention is directed to some characteristics of these cases which have been treated by us and studied by Vogel's method of detecting mercury in excreta.

In the first place, it has been found that mercury, in cases of mercuric chlorid poisoning, is regularly found in the stomach

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See page 183.

for several days after the ingestion of the poison. That it is not wholly swallowed in the saliva but is secreted by the stomach in an effort to eliminate it from the body, and that it is not part of the poison held in combination with the mucous membrane of the stomach, seem to be indicated by the fact that after several days the tests are negative, to become positive again before all the mercury is eliminated. This corresponds to the behavior of the tests conducted on urine and feces, late in the poisoning. Certain experiments are given below which indicate the same thing.

Another feature which is a determining factor in the length of time treatment should be carried on is the behavior of the mercury elimination after the patients, apparently, are entirely recovered. A reference to the summaries of the tests for mercury under the case histories will show the very long time it takes to free the system of mercury after mercuric chlorid poisoning. In Case 6, in which nephritis existed before the poisoning, in Case 7, in which successive doses were taken, and in Case 8, in which a large dose was taken, the excretion of mercury by the kidneys, the colon and the stomach lasted for days after all symptoms had cleared up. It has become quite apparent that treatment need not be kept up after the urine, and particularly the stools, begin to show negative results.

The treatment, as it is formulated at present for cases coming under observation early, is as follows: The first indication is to give the patient the whites of several eggs and then to wash out the stomach thoroughly. This has usually been done before the patients are admitted to the hospital. On admission, the stomach contents are expressed and examined for mercury, the stomach is thoroughly washed, and a pint of milk introduced. If no stomach contents are obtained before lavage, then the lavage water is examined for mercury. Urine passed spontaneously, or that obtained by catheter, is examined for mercury. The metal appears in the urine in from three to twenty-four hours after it has been swallowed. If more than a day has elapsed since the poisoning occurred, a stool should also be examined for the poison. If the first lavage does not allay the nausea and vomiting, it is repeated after an hour, and the following routine is begun as soon as the stomach will permit.

1. The patient is given every other hour 8 ounces of the fol-

lowing mixture: Potassium bitartrate, 1 dram; sugar, 1 dram; lactose, one-half ounce; lemon juice, 1 ounce; boiled water, 16 ounces. Eight ounces of milk are administered every alternate hour.

2. The drop method of rectal irrigation with a solution of potassium acetate, a dram to the pint, is given continuously. The amounts of urine secreted under this treatment are very large. In Case 8, 269 ounces were passed in twenty-four hours on the fourteenth day of treatment.

3. The stomach is washed out twice daily.

4. The colon is irrigated twice daily, in order to wash out whatever poison has been eliminated in that way.

5. The patient is given a daily sweat in a hot pack.

It is imperative to emphasize the necessity of keeping up the treatment with the colonic drip enterocylsis day and night without interruption. It entails discomfort for the patient, but the victims of accidental poisoning are always willing to do anything to recover from their plight, and the attempted suicide usually repents rapidly of his error, and the hope of his life being saved stimulates his patience and desire to cooperate.

In cases in which one single dose has been taken, after two negative examinations of the urine, on successive days, it seems legitimate to stop the treatment. In the fifth case, the urine was found to contain no mercury on at least two examinations before discharge. In view of the behavior of subsequent cases, which have been more thoroughly studied, it is probable that this patient excreted mercury for some time after leaving the hospital. For the less severe cases, a week may be a sufficient time for treatment. When large or successive doses have been taken, or when there is a preexisting kidney lesion, or when treatment begins several days after the poison is taken, longer periods of treatment, up to three weeks, are necessary.

It is interesting to consider how much damage the kidney suffers as a result of eliminating, even in a highly diluted form, an amount of mercury which hitherto in medical experience has led to fatal results. That such damage is not always great is indicated by the fact that in Case 5, 76 per cent. of phenolsulphonephthalein was excreted in two hours, the second day that the man's urine was free from mercury. In Case 3, the phenol-

sulphonephthalein excretion was 55 per cent. Six weeks after discharge the patient was apparently free from sequelæ of her poisoning.

With one exception all these cases came under treatment within twenty-four hours of taking the poison. In cases which have reached the stage of anuria, however, favorable results cannot always be expected. While it is perfectly possible to reestablish urination, the cases quite regularly go on to a fatal termination, with degenerative liver changes, and a colitis of the so-called diphtheritic type. In one of the present cases, Case 6, in which there was preexisting nephritis, the urinary output was reduced to 32 minims in twenty-four hours on the fifth day after taking the drug in spite of the fact that treatment was begun three hours after the poisoning. Recovery resulted, but the treatment was much longer than usual. Two of the patients were in the hospital over six weeks and gave positive Wassermann reactions, which were entirely unaffected by the poisoning.

Under the treatment detailed above, these patients quiet down to their routine and, as a rule, do not suffer except from the discomforts of the therapeutic measures. The stomach becomes tolerant of the milk diet and the alkaline drink after from twenty-four to thirty-six hours. The kidney secretion at first is excessive, and may run up to 130 or more ounces in the twenty-four hours. This usually diminishes in spite of the continued exhibition of fluid between the fifth to the tenth day, and may nearly stop altogether, as in Case 6. It is at this period that the continuous water cure and the rest in bed must be insisted on. If this period is successfully passed, the secretion of urine again increases often to a higher level than at first. The mercury itself seems to act as a diuretic at this stage of the treatment. The bowels usually show some irritation, but when the treatment is faithfully carried out, the tendency to diarrhea and colitis is regularly controlled by the colonic irrigations, and other medication for this symptom has been rarely necessary.

In order to clear up some of the toxicologic points which have been raised by the study of these cases, Professor Lieb has carried out the following experiments in the pharmacology laboratory at the College of Physicians and Surgeons: Several animals were operated on under narcosis, and after the esophagus had been

tied off so that no connection was left between the pharynx and the stomach, a hypodermic dose of mercuric chlorid was given. The excretion of the poison was then studied by the Vogel method. Three series of experiments were done. In Series 1, two rabbits were used. After the preliminary operation each received 20 mg. of mercuric chlorid hypodermically. After a few hours, mercury was found in the contents of the stomach and in that of the colon of both animals. In Series 2, three decerebrated cats were employed and prepared in the same way as given above. The presence of mercury was demonstrated in two of these animals in the urine and feces, and also in the stomachs after from four to eight hours. In Series 3, five decerebrated cats were again experimented on, and both the esophagus and the duodenum were tied off. From 15 to 20 mg. were injected into the jugular vein. Three cats gave a positive reaction after from six to eight hours. Another line of investigation concerning the elimination of mercury was made in the wards. Two patients who needed hot packs were receiving weekly hypodermic doses of mercuric salicylate. In both cases pads of filter paper were placed on the skin of the axilla, and after being saturated in the sweat of the patients, reacted positively to the Vogel test for mercury.

EXTRACT FROM URINE CHART, CASE 1

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
Dec. 9	720	Acid	.....	Trace	0	Many red blood cells
Dec. 10	840	Acid	.....	0	0	No blood (catheterized)
Dec. 11	900	Acid	1.030	Trace	0	Urates, no casts found
Dec. 12	540	Acid	.....	Trace	0	Pus cloud
Dec. 13	750	Acid	1.036	Trace	0	Pus sediment
Dec. 14	840	.....	.....	.....	..	
Dec. 15	540	Acid	1.030	Trace	0	Pus cloud
Dec. 16	420	Acid	1.030	Trace	0	Pus and blood sediment
Dec. 17	660	Acid	.....	.....	0	Pus and blood sediment
Dec. 18	540	Acid	1.014	Trace	0	Pus cloud
Dec. 19	420	Alkaline	1.035	Trace	0	Pus cloud
Dec. 20	420	Acid	.....	Trace	0	Few leukocytes and ep. cells
Dec. 21	960	Alkaline	1.026	Faint trace	0	Hyaline casts

CASE 1.—Admitted December 8. Three days before admission, patient had inserted two 7½-grain mercuric chlorid tablets in the vagina to relieve pain in the lower abdomen. Soon afterward she began to have severe abdominal pain, and her mouth became sore and salivated. She had vomited four times before admission.

The patient appeared ill and uncomfortable; the mouth was foul, with sordes on teeth and gums; tongue was badly coated; there was moderate salivation; tonsils and pharynx were congested, and the parotid gland on each side was swollen and tender. In the vagina there were yellow and dark brown sloughs on the inner side of each labium major, also yellow and white sloughs on both sides of vagina. The cervix was red and swollen, and there was a watery discharge from the os uteri.

December 9, saline douche was ordered twice daily, and gastric lavage was given, followed by castor oil, through the tube. Colon irrigation (4 gallons) was repeated twice daily.

December 23, patient's condition had decidedly improved in last few days; mouth was much cleaner; she still had a vaginal discharge. Patient left hospital against advice, and was reported well three years later.

CASE 2.—Admitted November 21. On the morning of the day of admission at 2 a. m., patient had inserted one 7½-grain mercuric chlorid tablet in vagina to avoid conception. Soon afterward she had severe burning sensation in that region and attempted douching, without success. Realizing the gravity of the situation, she came to the hospital, complaining less of pain in the vagina, than peculiar paresthesia and cramplike pains in hands and feet. No salivation was evident.

Physical examination was negative, except for a good deal of redness of vulva, with seropurulent discharge from vagina. Mucous membrane of vagina was covered with a white slough. Cervix was red.

Treatment was ordered as follows: an alkaline douche twice daily, forced fluid diet, a saline colon irrigation twice daily. The patient was discharged from the hospital at the end of a week.

EXTRACT FROM URINE CHART, CASE 2

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
Nov. 21	1,620	Acid	1.008	Faint trace	0	Many leukocytes; no casts
Nov. 22	3,390	Alkaline	1.006	Faint trace	0	Few hyaline casts
Nov. 23	4,320	Alkaline	1.008	Faint trace	0	Few hyaline casts
Nov. 24	3,990	Alkaline	1.008	Faint trace	0	Few hyaline casts
Nov. 25	4,920	Alkaline	1.014	Faint trace	0	

CASE 3.—Admitted February 12. Twenty-four hours previously she had swallowed three 7½-grain mercuric chlorid tablets in mistake for soda mints. Immediately she drank milk mixed with eggs, and a doctor arriving half an hour later washed out her stomach. She began to vomit soon after taking tablets, but this was relieved by the lavage. Six hours ago she began to have epigastric pain and abdominal cramps, accompanied by diarrhea. Urine was scanty and she had headache and sore throat.

EXTRACT FROM URINE CHART, CASE 3

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
Feb. 12	180	Acid	1.025	Marked trace	0	Few hyaline casts
Feb. 13	750	Neutral	1.022	Marked trace	0	Hyaline and gran. casts
Feb. 14	2,450	Alkaline	1.019	Marked trace	0	Hyaline and gran. casts
Feb. 15	1,500	Alkaline	1.013	Marked trace	0	Pus cloud
Feb. 16	400	Acid	1.022	Marked trace	0	Hyaline and gran. casts
Feb. 17	925	Neutral	1.021	Faint trace	+	Hyaline casts
Feb. 18	600	Alkaline	1.013	Faint trace	..	Hyaline casts
Feb. 19	900	Neutral	1.013	Faint trace	0	Leukocytes
Feb. 20	900	Neutral	1.016	Faint trace	0	Leukocytes
Feb. 21	950	Alkaline	1.017	Faint trace	0	Leukocytes
Feb. 22	1,800	Acid	1.011	Faint trace	0	Leukocytes
Feb. 23	1,500	Acid	1.012	Faint trace	0	Pus cloud
Feb. 24	2,400	Acid	1.014	Faint trace	0	Pus cloud
Feb. 25	800	Alkaline	1.015	Faint trace	0	Pus cloud
Feb. 26	400	Acid	1.012	Faint trace	0	Leukocytes
Feb. 27	550	Acid	1.015	Faint trace	0	Leukocytes
Feb. 28	425	Alkaline	1.016	Faint trace	0	Leukocytes

Phenolsulphonephthalein excretion, February 12, 51 per cent.
Phenolsulphonephthalein excretion, February 28, 55 per cent.

Physical examination was negative, except for general abdominal tenderness, most intense over lower half.

On admission the patient was put on a continuous rectal irrigation by the drop method, containing potassium acetate 1 dram to the pint. This was continued from February 12 to 17; patient then complained of soreness about rectum, and of the proctoclysis making her very nervous. She was then given a colon irrigation each day for four days, when the proctoclysis was again started, and continued until discharge. Hot packs were given daily while the patient was in the hospital. The lumbar region over the kidneys was cupped every three hours during the first three days she was in the hospital.

Patient left the hospital, February 28, in very good condition, and although advised to remain in hospital until urine was clear of albumin, she left against advice.

CASE 4. Admitted November 11. On night of admission, patient took two 7½-grain mercuric chlorid tablets. She arrived in the hospital in less than an hour, where stomach was washed. Past history was negative, except that one year before this she was treated for two months for "kidney trouble."

Physical examination revealed the right kidney distinctly palpable, but otherwise was negative.

November 11, mercury present in both vomitus and urine.

November 12, treatment was started on continuous rectal irrigation by the drop method, containing potassium acetate one-half ounce to quart of water.

November 18, patient left the hospital cured, although no examination for mercury was noted at time of discharge.

EXTRACT FROM URINE CHART, CASE 4

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
Nov. 11	.....	Acid	1.015	Trace	0	Many hyaline casts
Nov. 12	1,770	.....	.....	.....	..	No blood, no casts
Nov. 13	2,440	Acid	1.005	0	0	Few leukocytes
Nov. 14	4,070	Acid	1.005	0	0	Few leukocytes
Nov. 15	4,320	Acid	1.010	0	0	Few leukocytes
Nov. 16	3,630	Alkaline	1.010	Trace	0	Few leukocytes
Nov. 17	1,340	Alkaline	1.010	0	0	Few leukocytes
Nov. 18	.....	Acid	1.005	0	0	Few leukocytes

CASE 5.—Admitted January 23. About one hour before admission patient took two tablets which he thought were headache medicine. Ten minutes later he had burning pain in stomach and took a glass of milk which gave slight relief. The pain continued, and he came to hospital. He vomited in examining room and was given a gastric lavage (both of which proved to contain mercury). Patient brought a tablet of the medicine with him, which proved to be the ordinary 7½-grain tablet of mercuric chlorid. On being sent to ward, he was given another gastric lavage lasting fifteen minutes, then drank one pint of milk; one-half hour later the stomach was again washed clean (this is the lavage reported January 24 as containing mercury) He was put on milk diet, continuous proctoclysis (drop method) with potassium acetate, 1 dram to pint of water. Potassium bitartrate solution, 8 ounces, was ordered every hour. Patient was discharged, January 31.

EXTRACT FROM URINE CHART, CASE 5

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
Jan. 23	2,550	.....	1.008	Very faint trace	0	Few leukocytes and ep. cells
Jan. 25	2,200	Neutral	1.010	Faint trace	0	Few leukocytes and ep. cells
Jan. 26	3,100	Neutral	1.008	Faint trace	0	Many leukocytes and triple phos. crystals
Jan. 27	3,900	Alkaline	1.010	Faint trace	0	Few leukocytes and ep. cells
Jan. 28	1,025	Neutral	1.010	Very faint trace	0	Few leukocytes
Jan. 29	1,950	Alkaline	1.010	Faint trace	0	Few leukocytes and ep. cells
Jan. 30	2,350	Neutral	1.010	Faint trace	0	Few leukocytes and ep. cells
Jan. 31	600	Acid	1.020	Very faint trace	0	Few leukocytes and ep. cells

January 29, phenolsulphonephthalein test, first hour, 50 per cent.; second hour, 26.5 per cent.; total, 76.5 per cent.

Summary of Tests for Mercury, Case 5

	Jan. 23	Jan. 24	Jan. 25	Jan. 26	Jan. 27	Jan. 28	Jan. 29
Urine	Positive	Positive	Positive	Positive	Positive	Negative	Negative
Vomitus	Positive	.....	.....	.....	.....	.....	.....
Lavage	Positive	Positive	.....	.....	.....	.....	.....
Stool	.....	Positive	Positive	.....	.....	.....	.....

Case 6. —Admitted March 26, at 5:45 p. m. At 3 p. m., patient took four 7½-grain mercuric chlorid tablets. Shortly after this she took some castor oil and mustard water which made her vomit. At 4 o'clock she was seen by a physician who gave her a thorough gastric lavage and some milk.

The past hisory of the patient revealed that she had had an interstitial keratitis and was given two doses of salvarsan and a prolonged mercurial treatment. One other sister had congenital syphilis. The Wassermann reaction is said to be positive in both her sister and herself.

Physical examination was unimportant. The routine treatment as outlined in this article was carried out beginning March 26 and continued for more than five weeks. March 26, the vomitus and lavage water were both positive for mercury. The stools, the urine and the gastric lavage were positive to the mercury tests for a period of several weeks. March 28, the first negative test occurred in the gastric lavage. May 2, the first negative test was found in the urine. May 16, the stool was negative for the first time. The mercury was not completely eliminated by July 3 and occasional positive results were found up to that date, when the examinations were stopped.

Extract from Urine Chart, Case 6

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
Mar. 27	396	Acid	1.010	Faint trace	0	Hyaline and gran. casts
Mar. 28	137	.....	.....	....	..	
Mar. 29	45	.....	.....	....	..	
Mar. 30	45	.....	.....	....	..	
Mar. 31	2	.....	.....	....	..	
Apr. 1	55	.....	.....	....	..	
Apr. 2	396	.....	.....	....	..	
Apr. 3	853	.....	.....	....	..	
Apr. 6	1,462	Neutral	1.010	Faint trace	0	Few leukocytes, hyaline casts
Apr. 11	660	Alkaline	1.015	Marked trace	0	Few leukocytes
Apr. 13	1,310	Neutral	1.010	Trace	0	Pus cells
Apr. 30	3,991	Alkaline	1.002	Very faint trace	0	Few leukocytes
May 10	4,640	Neutral	1.005	Very faint trace	..	Few leukocytes

The striking feature in this case is that on March 31 there was an almost complete suppression, when the twenty-four-hour secretion was only 32 minims. March 26, the Wassermann reaction was double plus, and on April 24 it was four plus.

The phenolsulphonephthalein excretion in two hours was as follows: April 7, 3.3 per cent.; April 9, 4 per cent.; April 17, 41 per cent.; April 23, 47.5 per cent.; May 10, 71.4 per cent.

Case 7.—Admitted April 9. Four days ago patient took one 7½-grain mercuric chlorid tablet. The only symptom complained of is that she vomited a little later. Twenty-four hours ago, she took two more 7½-grain tablets, and about an hour later had pain in the abdomen for which she took some "soda." After this she vomited several times. About 3 o'clock this afternoon, she swallowed another 7½-grain mercuric chlorid tablet, and vomited about half an hour later. The bowels have moved several times a day for the last few days, but she has felt fairly well since taking the first tablet except for a burning sensation in abdomen, and headache, and has had no appetite. She has noticed no decrease in amount, or change in character of urine.

Physical examination was unimportant. The routine treatment was given and continued for two months, although the symptoms of poisoning were practically absent after the first days of gastric irritation. The tests for mercury were positive for many days both in the urine and the stools and the gastric lavage. The first negative examination in the urine was on April 27, and in the feces on June 8. But the mercury was not completely eliminated by July 3. Occasional positive results were obtained up to that date, when the examinations were stopped.

Extract from Urine Chart, Case 7

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
Apr. 10	1,175	Acid	1.010	Faint trace	0	Many leukocytes
Apr. 12	600	Alkaline	1.010	Very faint trace	0	Many leukocytes
Apr. 13	1,600	Neutral	1.015	Faint trace	0	Many leukocytes; mucus
Apr. 17	3,500	Alkaline	1.005	Trace	0	Many leukocytes
Apr. 30	.....	Alkaline	1.010	Very faint trace	0	Few leukocytes
May 10	.....	Neutral	1.015	Very faint trace	0	Many leukocytes
May 20	1,500	Neutral	1.010	Faint trace	0	Many leukocytes
May 22	.....	Neutral	1.010	Trace	0	Many leukocytes
June 7	.....	Acid	1.005	Very faint trace	0	Many leukocytes
June 22	.....	Neutral	1.005	Faint trace	0	Many leukocytes
June 23	1,800	Neutral	1.015	Trace	0	Few leukocytes
June 28	.....	Alkaline	1.005	Trace	0	Few leukocytes
July 1	.....	Acid	1.015	Trace	0	Few leukocytes

The phenolsulphonephthalein excreted in two hours was as follows: April 9, 66.5 per cent.; April 21, 62.5 per cent.; June 22, 65 per cent.

Case 8.—Admitted June 14. About an hour and a half before admission to the hospital, patient took by mistake six 7½-grain mercuric chlorid tablets,

dissolved in water, in mistake for a sleeping mixture. She drank a glass and a half of water after this. In a few minutes she felt great discomfort in her throat, and vomited a large amount. She was given three raw eggs, a doctor was called and she was sent to the hospital. Her physical examination was negative. The symptoms need not be given here. The routine treatment was instituted.

June 14, the urine was positive to the mercury test, but the gastric lavage on admission was twice negative. It became positive on the 15th, and remained so until the 17th, after whch it was negative. June 23, the urine was negative for the first time, and on July 20 the first negative stool was examined. The elimination of mercury was not complete at time of discharge, July 28.

EXTRACT FROM URINE CHART, CASE 8

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
June 14	1,098	Alkaline	1.025	Very faint trace	0	Mucus
June 16	2,650	Alkaline	1.005	Very faint trace	0	Many leukocytes
June 22	5,667	Neutral	1.005	Faint trace	0	Few leukocytes
June 24	4,692	Alkaline	1.005	Very faint trace	0	Few leukocytes
June 28	8,196	Alkaline	1.010	0	0	Few leukocytes
July 1	.....	Alkaline	1.016	Trace	0	Few leukocytes, hyaline and gran. casts
July 4	5,911	Alkaline	1.005	0	0	Few leukocytes
July 6	6,764	Alkaline	1.005	Faint trace	0	Few leukocytes
July 9	.....	Alkaline	1.020	Very faint trace	0	Few leukocytes
July 11	5,484	Alkaline	1.005	0	0	Few leukocytes
July 14	.....	Alkaline	1.005	0	0	Few leukocytes
July 17	4,916	Alkaline	1.010	0	0	Few leukocytes
July 19	4,052	Alkaline	1.005	0	0	Few leukocytes
July 23	.....	Alkaline	1.005	Faint trace	0	Few leukocytes

June 14, the phenolsulphonephthalein excretion in two hours was 55.5 per cent., and on July 14, it was 45.7 per cent. July 14, the Wassermann reaction was two plus in spite of the excessive dose of the mercury.

CASE 9. —Admitted July 11. About three hours before admission, patient took one 7½-grain tablet of mercuric chlorid by mistake for aspirin. He went to a doctor's office immediately and in about fifteen minutes was given mustard and vomited several times. The stomach was then washed out thoroughly and patient drank milk. After that he was sent to the hospital. Distress in abdomen followed the lavage, but there was no actual pain. The bowels were regular, the urination was normal, the appetite good.

Physical examination was negative and the routine treatment was begun. July 11, the urine, stool, vomitus and lavage were all positive to mercury. July 24, the first negative urine was found, but there were no negative stools before leaving the hospital.

Extract from Urine Chart, Case 9

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
July 11	1,950	Acid	1.010	Very faint trace	0	Few ep. cells, hyaline cast
July 12	3,930	Neutral	1.005	Faint trace	0	Few ep. cells, hyaline cast gran. casts
July 13	3,656	Neutral	1.005	0	0	Epithelium
July 14	4,204	Acid	1.010	Very faint trace	0	Epithelium
July 15	2,833	Acid	1.015	Very faint trace	0	Epithelium
July 16	3,900	Acid	1.010	Very faint trace	0	Epithelium
July 17	3,929	Acid	1.013	Faint trace	0	Epithelium
July 18	4,288	Acid	1.010	Faint trace	0	Epithelium, occasional gran. cast
July 19	2,986	Neutral	1.005	Very faint trace	0	Epithelium and mucus
July 20	4,022	Neutral	1.005	Very faint trace	0	Epithelium and mucus
July 21	2,376	Neutral	1.005	Very faint trace	0	Epithelium and mucus and leukocytes
July 23	3,686	Neutral	1.005	Very faint trace	0	Epithelium and mucus and leukocytes
July 24	.....	Alkaline	1.020	Faint trace	..	Epithelium and mucus and leukocytes

July 24, phenolsulphonephthalein test showed 55.8 per cent. excretion in two hours.

Case 10.—Admitted March 21. Patient took by mistake three tablets of mercuric chlorid dissolved in 6 ounces of water, as a gargle, and swallowed practically all of it. She vomited fifteen minutes later a greenish fluid. Pain in the epigastrium followed immediately, and patient came at once to the hospital where she vomited again, a thick, foamy, colorless mucus. The patient came under treatment within an hour after poisoning. There were no symptoms after the first two days, and the treatment was routine. She was discharged March 31. All the excreta and stomach washings were positive to mercury for three days. The stomach washing was first negative on March 24, to be again positive March 27. The urine was first negative on March 24 and positive at intervals until treatment ceased, March 31. The colon irrigations were positive until March 30.

Extract from Urine Chart, Case 10

Date	Amount, c.c.	Reaction	Sp. Gr.	Albumin	Sugar	Microscopic
Mar. 22	2,737	Acid	1.025	Faint trace	0	Pus cloud
Mar. 24	3,107	Alkaline	1.010	Faint trace	0	Many leukocytes
Mar. 26	3,107	Alkaline	1.010	Very faint trace	0	Occasional gran. cast
Mar. 30	3,778	Acid	1.010	Very faint trace	0	No casts

A REPORT OF A SERIES OF PNEUMOCOCCIC PNEUMONIAS, CLASSIFIED ACCORDING TO THE GROUP OF TYPE OF PNEUMOCOCCUS PRESENT

LEWIS F. FRISSELL, M. D., and L. W. FAMULENER, M. D.

The following report is the result of work done at St. Luke's Hospital in the differentiation of the types of pneumococcus in sputum from cases of acute lobar pneumonia during the late winter, spring, and summer of 1915. Since most of the cases observed were admitted during the spring and summer months, a comparison of these findings with those of other workers collected during the winter months chiefly might be of interest. However, owing to the small number of cases studied, no definite conclusions can be derived from this series. In all, some eighteen cases were seen, including a re-infection in one patient, which gave definite laboratory results. A few additional cases were excluded, as the agglutination tests gave questionable results. Based upon the laboratory tests, the cases may be divided as follows:

Group I	9	cases
Group II	7	"
Group III	0	"
Group IV	2	"
Total	18	"

An effort was made to separate these cases into four clinical groups—mild, moderately severe, severe, and fatal—with a view to gaining some insight into the type of infection produced by the respective groups. Thus classified, the following scheme shows their distribution:

	Group I	II	III	IV
Mild		1		
Moderately severe	5	1		1
Severe	3	1		
Fatal	1	4		*1

Those cases occurring during the summer months were infections with organisms either of Group I or of Group II.

One very interesting case is No. 105103. In this the original infection was caused by pneumococcus Group I, and the disease ran a severe course ending in recovery; a few days, later, however, the patient developed a Group IV infection, which terminated in empyema and death. This suggests the possibility that reinfections in pneumonia, which we ordinarily consider relapses, may possibly be due to a new infection with an organism of another group.

Our thanks are due to the staff of the Rockefeller Hospital for its courtesy in supplying specific agglutinating sera for pneumococcus Groups I and II, and for instruction in technique, and also to Doctors Hollis and Lambert, from whose services the cases were taken. Acknowledgment is due to Miss Julia Hewitt for assistance in carrying out the tests.

PROTOCOL OF INDIVIDUAL CASES

Hospital Case No.	Date of Admission	Date of test	Pneumococcus Group	Clinical Type
104969	March 8	March 10	II	Fatal
105101	March 14	March 16	I	Moderately severe
*105103	March 14	March 16	I	Severe
105155	March 16	March 17	II	Moderately severe
105254	March 22	March 23	I	Moderately severe
*105103	March 14	April 5	IV	†Fatal
105641	April 11	April 12	II	Mild
105706	April 14	April 18	I	Moderately severe
105672	April 12	April 19	I	Moderately severe
105906	April 23	April 25	IV	Moderately severe
105949	April 26	April 27	II	Fatal
105936	April 25	April 27	I	Moderately severe
106507	May 25	May 26	II	Fatal
106573	May 28	May 29	II	Severe
106782	June 8	June 9	I	Fatal
106936	June 15	June 17	I	Severe
107675	July 26	July 28	I	Severe
107890	August 6	August 9	II	Fatal

* Same case.

† Death due to empyema and exhaustion.

NITROGEN RETENTION IN NEPHRITIS

LEWIS F. FRISSELL, M.D., AND KARL M. VOGEL, M.D.

The following is a report of a series of non-protein nitrogen determinations, made at St. Luke's Hospital during the spring and summer of 1915, on the blood of patients suffering from nephritic, cardiac, and vascular conditions and a wide variety of miscellaneous disorders. A phenolsulphonephthalein test, also was done in most instances, for the purpose of determining, if possible, which of the two methods afforded a more reliable index of kidney function and which of the two had the greater prognostic value.

The phthalein test was carried out according to the technique of Rowntree and Geraghty; the other determinations were made after methods based on those of Folin and Denis. The one procedure uses as its index the power of the kidney to excrete a given dye in a known length of time; while the other measures the accumulation in the blood of the unexcreted non-coagulable nitrogen. The conclusions of Fitz[1] are: "The amylase and phenolsulphonephthalein tests show the renal function at the moment; the blood nitrogen test is more influenced by the duration of the condition causing its accumulation." Circulatory conditions would therefore be more apt to influence the phthalein than the nitrogen tests.

The patients were in most cases on a low protein diet and the blood was withdrawn before the subject had breakfasted. The cases may be divided into four groups:

A. Nephritic
B. Cardiac
C. Cardionephritic
D. Miscellaneous

[1] Fitz. Arch. Int. Med., 1915, xv, 524.

GROUP A

Nephritic

Case No.	Non-protein Nitrogen	Urea	Uric Acid	Phthalein	
2	30.0	.....	0.24	.....	Uremia, fatal
4	28.0	.....	....	.....	Uremia, fatal
5	28.8	.....	....	.....	
7	.....	.....	3.3	48.7	
19 May 4	40.5	22.5	....	41.	
June 10	32.5	16.25	2.5	28.8	
July 4	43.8	.....	....	28.	
20	46.28	21.75	....	65.6	
24	45.5	11.75	....	.....	
25	76.5	42.75	....	7.7	Hemiplegia
34	89.0	46.5	....	Too small	Uremia
35	25.0	12.5	....	35.7	
38	125.0	.....	....	50.	Uremia
41	53.0	21.7	....	5.16	
42	23.0	13.0	....	51.13	
46	20.0	11.5	3.8	55.8	
31	21.85	11.	....	81.9	
48	41.2	19.5	....	71.4	
49	39.	15.5	....	66.7	Pyelonephritis
50	53.0	26.5	....	.....	
57	54.	24.75	....	27.5	
76	.....	17.5	....	83.5	
78	.....	12.50	....	71.	
79	.....	15.25	....	44.	
80	31.	.....	....	.....	
81	60.	.....	....	Too small to estimate	
83	.....	15.6	....	45.5	
88	50.	33.0	....	Trace	Uremia
93	31.2	12.	....	45.5	
98	25.	14.	....	70.5	
100	43.	22.	....	25.	Uremia
101	55.	29.	....	50.	
107	20.	11.	2.85	55.	

GROUP B

Cardiac

Case No.	Non-protein Nitrogen	Urea	Uric Acid	Phthalein	
27	22.2	.....	....	55.6	
47	21.85	12.0	....	38.1	Aortitis, specific
56	23.0	10.6	....	38.5	
77	.....	21.75	....	38.5	
89	16.0	9.0	....	60.0	
99	36.0	19.0	....	65.0	
106	20.0	14.0	....	73.5	

GROUP C

Cardionephritic

Case No.	Non-protein Nitrogen	Urea	Uric Acid	Phthalein
18 May 4	33.	16.	....	16.2
June 8	40.	18.	....	36.
July 6	30.	17.	....	20.
21	51.5	37.5	....	75.6
39	68.	.....	....	62.5
40	66.	.....	....	28.6
52	32.	45.	1.0	59.1
84	31.5	14.7	....	78.

GROUP D

Miscellaneous

Case No.	Non-protein Nitrogen	Urea	Uric Acid	Phthalein	
6	18.03	.....	....	....	Post-pneumonic
9	17.8	.....	....	89.7	Pneumonia
10	18.6	.....	....	58.2	Pylorospasm
11	11.8	.....	....	97.6	Rheumatism
12	13.8	.....	....	95.4	Rheumatism
13	10.9	.....	....	90.5	Pityriasis
14	20.8	.....	....	85.7	Post-pneumonic
15	32.6	15.3	....	67.6	Rheumatism
16	32.5	14.0	....	96.9	Gastritis
17	16.8	.....	....	95.9	Diabetes
22	32.5	17.25	....	....	Anemia
26	24.2	10.35	....	42.85	Gastric ulcer
28	25.0	10.01	....	87.5	Tonsillitis
29	27.0	14.75	....	....	Pleurisy
30	28.5	13.2	....	84.3	Rheumatism
32	24.0	.....	.....	72.7	Carcinoma
33	23.0	.....	....	80.5	Tuberculosis
36	22.5	10.1	....	40.8	Flat foot
37	21.5	7.75	.....	71.2	Hyperthyroidism
43	29.5	15.0	.....	57.14	Diabetes
44	19.0	11.6	.....	58.5	Pregnancy, vomiting
45	27.0	15.5	....	95.4	Tumor of cord
55	33.5	15.0	....	....	Hyperthyroidism
58	21.0	9.8	....	47.0	Plumbism
59	30.5	14.0	....	....	Pernicious anemia
71	28.0	.....	1.7	....	Optic Atrophy
73	29.5	.....	....	62.5	Cirrhosis of liver
75	.....	.....	0.86	35.5	Rheumatism
82	.....	12.5	....	....	Diabetes
85	45.5	.....	....	....	Scleroderma
86	35.5	.....	....	92.0	Malingerer
90	18.0	8.0	....	91.0	Hodgkin's disease
91	23.0	13.	2.8	85.4	Sciatica
102	28.0	14.	5.0	....	Arthritis
103	20.0	10.4	2.25	50.0	Arthritis
104	21.0	14.	1.25	....	Arthritis
105	27.0	10.5	3.3	....	Arthritis

The miscellaneous cases, with only one or two exceptions, showed no noteworthy alteration in either nitrogen retention or phthalein excretion.

The nephritic group, as one would expect, showed unaltered values for both tests in many cases, a retention of nitrogen in the more severe cases associated with a diminished phthalein output: while the uremic cases showed both a marked increase in nitrogen values and a diminution in the amount of dye eliminated. The most interesting cases perhaps, are in the cardiac group. One patient (18) suffering from myocarditis and endocarditis clinically suggested uremia rather than a cardiac condition, but while the phthalein output was low, there was only slight retention of nitrogen. Post-mortem examination, however, revealed kidneys with marked congestion and slight nephritis and a very bad heart muscle with valvular disease.

In conclusion, though the number of cases in our series is too small to be in any way conclusive, our results seem to be in agreement with those of Fitz, and we feel that tests of nitrogen retention are a valuable aid in the determination of insufficient kidney function lasting over a considerable length of time, high values always suggesting grave renal involvement.

THE MEDICAL TREATMENT OF CHRONIC INTESTINAL STASIS*

W. A. BASTEDO, M. D.

Constipation has appeared under a new descriptive name, viz., intestinal stasis; but it is the recognition of the fact that poisoning may result therefrom that has made constipation and its treatment the subject of renewed interest. Constipation is not necessarily a condition of infrequent defecation, but may be accompanied by one or more daily stools and even diarrhea. This is seen (1) in the so-called "cumulative constipation," in which in spite of daily defecation there is a gradual feeling of accumulation and great benefit from a weekly cathartic; (2) in fecal impaction or fecal tumor, through or around which material finds a passage, though much retarded, or the irritation of which causes diarrhea; (3) in Boas' "fragmentary constipation," in which there is a constant consciousness of feces in the rectum, but no relief from repeated attempts at defecation, these resulting merely in the passage of one or two fragments now and then, and (4) in the condition in which, in spite of one or more daily stools, occasional hard, foul-smelling masses are passed.

A review of the treatment of chronic constipation might very properly be preceded by a discussion of the causative factors of the condition; but I shall omit this discussion, because I intend to confine my remarks to the treatment of those cases of chronic stasis with no discovered anatomic or pathologic condition to which the stasis may be attributed. These constitute the great majority of stasis cases, and are seen every day by the physician.

Measures for relief in such cases of chronic intestinal stasis include: the formation of good defecation habits, prompt response

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to the desire to defecate, exercise, massage, support of the abdominopelvic walls, foods, water-drinking, softening agents which increase the bulk of the feces, cathartics, suppositories, enemas and surgical intervention.

1. *Habit Formation*—This is the establishment of a regular time for the daily stool. The best time would seem to be just after breakfast, because the morning activities and the taking of food both favor colon peristalsis. By the Roentgen ray Hertz noted a perceptible advancement of the colon contents after each meal, and this was most marked after breakfast. Cash noted the increased movement after exercise. Even when there is no desire to defecate, the attempt should be made; for the voluntary effort may force some feces into the rectum and so result in the regular defecation reflexes, and the passage of a stool. Good habits also include proper attention to the act itself, for failure to defecate may result from the concentration of the mind elsewhere, as on some interesting book or newspaper.

2. *Response to Desire*—In those who do not go to the water-closet when the desire comes, the feeling tends to pass away, and the fecal matter remains in the rectum without arousing further desire to empty the bowels. If this is often repeated the rectum loses its sensitiveness. Hence, rectal insensitiveness is a cause of intestinal stasis in those who habitually suppress the desire to defecate. Such are dentists and other professional people, women in business who have to pass a number of men to reach the water-closet, those who have access to filthy closets only or are subject to inadequate facilities, etc.

3. *Exercise.*—It is generally understood that bodily activity is sufficient to ensure good bowel movements. Yet we have encountered a number of athletes and active persons who were constipated, and some who led very sedentary lives and yet had daily copious stools (the latter are usually large eaters). Other things being equal, however, exercise tends to promote defecation, both by creating rapid changes in intra-abdominal pressure, and by increasing the appetite so that more food is taken and the food residue in the bowel correspondingly increased. Any active exercise may be prescribed, such as brisk walking, running, rowing, horseback-riding, tennis and golf, all of which are done in the open air, or dancing and gymnasium and home exercises, which

are taken indoors. Simple daily exercises of benefit may be taken in the lying or standing position. Those to be taken in the lying position might be: lying on the back and raising the legs, one at a time and both together to a right angle with the trunk, raising the trunk to a right angle with the legs, etc. Those taken in the standing position might be: bending forward, knees stiff, till the fingers touch the floor, and then backward as far as possible, the hands being raised above the head; bending far over to one side, and then to the other; rotating the body on the hips, the arms being extended; alternately raising the knees till the thigh hits the abdomen, etc. These exercises should be performed in a quick and positive manner, and not in a dilatory way. Troublesome exercises, and difficult postures such as the Trendelenburg and knee-chest, are seldom persisted in by the patient for any length of time.

4. *Massage.*—This may be a helpful measure when the manipulation of the abdomen is done by some one with general anatomic knowledge and special information about the abdominal organs of the patient under treatment. It should not be instituted if there is any suspicion of a duodenal ulcer, a chronic appendicitis or other inflammatory condition. The bladder should always be empty. The massage may be either superficial or deep. It may be performed by kneading, by a rotary motion in which the abdominal wall slips over its contents, by a rapid vibratory motion, or by rapid slapping with the edge or flat side of the hands, always with a general movement in the direction of the colon. It may be given with a mechanical vibrator. Self-massage may be carried on by rolling a cannon ball, or ball of clay covered with chamois skin or leather, or a wooden ball filled with shot, over the abdomen in the direction of the colon.

5. *Support of the Abdominopelvic Walls.*—In defecation, normally the anterior abdominal muscles, the diaphragm and the pelvic floor contract or are set so as to raise intra-abdominal pressure. In most cases with ptosis of the viscera, however, not only do the weak abdominal muscles fail to make pressure, but also, by allowing the viscera to fall away from the diaphragm, they deprive this latter of its usual resistance. It is therefore difficult to raise intra-abdominal pressure. Hence an inelastic support extending around the body and from navel to symphysis

should be applied the first thing in the morning and worn till bedtime. In addition, if there is laxity of the pelvic floor, this should be attended to by a pessary or the necessary surgery.

6. *Foods.*—So far as their effect on the bowel movements is concerned, foods might be classed according to the chemical substances they contain, and the amount and character of their residue. They tend to promote peristalsis: (1) by increasing the bulk of the intestinal contents, as by their structural parts such as cellulose, skins, etc., and by unabsorbed oils and fats; (2) by mechanical irritation, as by seeds or husks; (3) by chemical action, as by sugars and fruit acids and their salts, and digestive products, such as soaps, aminoacids, etc.

It goes without saying that those who habitually undereat will be constipated; in fact, simple undereating is a frequent cause of constipation. The same is true of those who habitually eat food that is too readily digested. In either case the residue is insufficient. Therefore, abundant mixed meals usually favor bowel movements. For residue, cellulose, as furnished by fruits, vegetables, and other plant products is of very great importance; in fact, Rubener states that in the absence of cellulose almost everything is absorbed. Substances rich in cellulose pass into the intestines as paste and stimulate peristalsis, while meat, eggs and milk pass along as liquids, and so favor segmentation, but not peristalsis (Cohnheim). The products of the latter type are then rapidly absorbed and leave but little residue. Hertz reports that of the dry substance of meat, eggs, white bread and rice, only 5 per cent. appeared in the feces; while of the dry substance of green vegetables and brown bread 15 per cent., and of the dry substance of carrots and turnips, 20 per cent. was recovered. The feces of a mixed diet gave 100 gm. of water and 35 gm. of dry substance; the feces of a vegetable diet, 260 gm. of water and 75 gm. of dry substance.

Vegetables and salads mostly contain much fibrous tissue and cellulose. Many of them should be quite as much esteemed for the residue they leave for the bowels as for the nutritive matter they furnish for absorption. Such are lettuce, spinach, carrots, parsnips, oyster-plant, turnips, cabbage, cauliflower and squash. The salad dressing may be a means of furnishing abundant oil, which tends to be laxative.

Farinaceous foods include the cereals, the starchy vegetables, such as potatoes, and foods made with flour, such as bread. The cereals contain more or less cellulose. Oatmeal is especially laxative, because of the presence of indigestible husks and excessive cellulose and fat. It is often taken in the form of crackers. White bread leaves very little residue, but bread, biscuits and crackers made of graham flour, or containing bran, leave a large residue and tend to be laxative. It is to be remembered that graham flour is made by grinding the whole of the wheat kernel, bran and all, and is not bolted, so that it contains coarse particles. The so-called whole-wheat or entire-wheat flours have a portion of the bran removed and are bolted, but are coarser than white flour.[1]

Fats tend to be laxative largely because of the soaps which are formed from them in the intestines. They may be given in the form of cream, butter, olive oil, etc. It goes without saying that the greater their nutritive powers the less is their laxative effect; for if they are absorbed they do not pass on to the colon.

Fruits contain sugar, cathartic acids or salts, indigestible structural parts (fiber, cellulose, skins, etc.), seeds and non-absorbable colloid pectin bodies. Those most frequently considered laxative are prunes, figs and dates; but an apple, an orange or some grapes at bedtime or before breakfast will often insure the morning stool. Of this latter group, the acids and salts belong to the saline cathartic class. In young children one or two teaspoonfuls of orange-juice may be an effective stimulant to the bowel, its acid changing in the duodenum to the laxative sodium citrate.

Fruits and other coarse foods have the great disadvantage that they tend to promote gastric hyperacidity and flatulence. In obstructive or spastic types of constipation they may impede the action of the bowels, or may even form a food ball in the intestines. In one case, Coerr[2] removed from the ileum a ball which weighed 945 gm. and was composed of cereal husks, fruit seeds and other plant parts. In a number of instances we have found that the bowels acted better when the patient stopped fruit-eating.

7. *Water-Drinking.*—In those who live on a dry diet constipa-

1. Bull. 143, U. S. Dept. Agric.
2. Coerr, F. D. H.: Enteroliths, Jour. Am. Med. Assn., 1913, lxi, 2238.

tion is the rule; but the converse is not true, for the ingestion of large quantities of water does not make the bowels move. In two normal cases and six cases with chronic nephritis, we observed the effects of the administration of 8 ounces of water each hour for fifteen hours daily for one week. That was 7½ pints of water a day by mouth. In the normal cases the only noticeable effect was increased urination, the bowels not showing any increased activity. In the kidney cases the only observed effect was edema, neither the kidneys nor the bowels being appreciably increased in activity. As a matter of fact any excess of water is normally absorbed from the intestines with rapidity, and in any but kidney cases it serves to activate the kidneys rather than the bowels. Such absorption of water may, however, be greatly retarded by the solution in the water of salts containing poorly absorbable ions, such as the saline cathartics, or by substances in the digestive canal that hold the water, such as agar-agar (See next paragraph). And since, in order to keep the feces of a soft consistency, the food residue must hold a certain amount of water, the drinking of a reasonable amount of fluid should be prescribed for those who habitually underdrink.

8. *Medicinal Agents Which Give Bulk and Soft Consistency to the Feces.*—The more commonly employed ones are: agar-agar, liquid paraffin, and small (not expulsive) doses of the saline cathartics.

1. Agar-agar absorbs water and increases greatly in bulk, so that it softens and enlarges the fecal mass. It is not absorbed unless changed bacterially, but it has the disadvantage that if it is not carried out, it forms a most excellent culture medium for the growth of intestinal bacteria. It is not a very effective laxative and usually requires the addition of a little phenolphthalein or cascara. Used in a similar manner are flaxseed and psyllium seeds, which absorb water, become mucilaginous, and increase the fecal bulk. They serve the purpose of making the bowels move, but they are very subject to fermentation, with the development of gas in the intestines.

2. Liquid paraffin (petrolatum liquidum) is a remedy that has been extensively employed since it was called to the notice of the profession by Sir Arbuthnot Lane. It is a viscous liquid that is not at all changed chemically and not at all absorbed from the

alimentary tract, as shown by Bradley, 1911, and by Bloor, 1913, hence it serves to soften and to increase the bulk of the feces. It is also believed to be strictly non-irritant. Whether or not it exerts in the bowel a disinfectant effect on some of the strains of fecal bacteria has not been demonstrated. Finch and others have shown that it lacks disinfectant effect, but that, by virtue of its viscous and oily nature, it acts as an agent which retards the growth of bacteria implanted in it, but this does not serve as a test of its antibacterial value when mixed with food residue in the ileum or colon.

The refined oil is almost tasteless, is readily swallowed without any added flavor, and in doses of from 1 to 3 ounces a day is a mild but often efficient laxative. On account of its oily nature, it should not be taken just after meals, but preferably when the stomach is empty, that is, about an hour before lunch and dinner and at bedtime, for the oil will retard gastric digestion and prolong the emptying time of the stomach. In some cases, especially those in which the stomach-emptying is already retarded, the oil is not well tolerated, causes nausea or regurgitation, and has to be stopped. In others of the chronically constipated, the oil leaks from the anus so that it greases the clothes, yet without giving a proper stool; or it passes down as free oil without admixture with the food residue. In these cases reduction of the dose of oil and persistence in its use may meet with the desired result, but a small dose of another laxative, such as cascara, may be necessary.

On account of its recent extensive use as an internal remedy, a few words in regard to the quality and properties of this oil may be in order. It is quite generally asserted that the Russian oil is superior to the American for two reasons: (1) the absence of the harder paraffins, and (2) the absence of members of the olefin series of unsaturated hydrocarbons. But in the best American oils the paraffins are removed by chilling, and according to Sir Boverton Redwood, in his extensive work on petroleum, there is no sufficient evidence that the unsaturated hydrocarbons of Pennsylvania petroleum are olefins, and that, in any case, if there are any present they are readily removable by strong sulphuric acid. Redwood further states that "comparison of results obtained with American, Galician and Russian petroleum shows that the

same classes of hydrocarbons—the paraffins, the polymethylenes or naphthenes, and the aromatic hydrocarbons—are present in the petroleum from all three sources, but that the relative amount of naphthenes and in all probability of aromatic hydrocarbons is greatest in Russian and least in American petroleum." Marcusson[3] claims that, of stable saturated hydrocarbons which react with fuming nitric acid, the Russian oils contain a higher percentage than the American oils. There is obviously some difference between the Russian and American oils, but in the well-refined products the difference is not marked.

As a matter of fact, after a certain fraction of petroleum of the specific gravity desired has been obtained by repeated fractional distillations, the regular process of refining is to subject it to the action of strong sulphuric acid, then caustic alkali, and then water for thorough washing. A stream of dry air carries off the water. In some instances the oil is filtered through diatomaceous earth or bone-black. This refining process must be repeated many times, indeed as often as fifteen or sixteen times, perhaps, and it correspondingly increases the expense of production.

The proposed text for the Ninth Revision of the U. S. Pharmacopeia describes "liquid paraffin" as free from fluorescence, without odor or taste, and not acted on or colored by concentrated sulphuric acid or nitric acid in the cold. The British Pharmacopeia specifies that when the oil is mixed with an equal volume of sulphuric acid in a test-tube and placed in boiling water for ten minutes with frequent agitation, the color of the separated layer of acid should not be of a deeper tint than pale brown.

I have tested a number of oils on the market. Of nine samples which have been mixed with an equal volume of sulphuric acid, a portion not having been heated, and a portion having been heated on a waterbath for ten minutes, nearly all fail to come up to the requirements. Three of these samples are American and are fluorescent. The others are sold as Russian oil. Obviously the oils on the market are of very poor quality.

We do not yet know whether, when used internally, an oil is or is not harmful if it is not fully refined: but till we do know, it is the part of wisdom to demand only the very best.

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3. Marcusson: Chem. Ztschr., 1912.

In regard to the specific gravity of these oils, it would seem that the more viscous oils, those of specific gravity from 0.880 to 0.890, are the ones of choice. I have been of the belief that lighter oils than this were likely to pass through the bowel without satisfactory admixture with the feces; but in a study of this subject, I find that there is no rule about it, and that at times in certain patients, the heavier oils as well as the lighter will give a stool of pure oil or will leak from the anus. The question of the requirement of a high specific gravity must, therefore, remain an open one.

The more solid paraffin preparations, such as white petrolatum, have also been employed in the form of a flavored and usually colored jelly. They are apparently just as active as the liquid preparations.

3. Of the saline cathartics, the softening doses are smaller than the usual expulsive doses; for instance, 1 or 2 drams of magnesium sulphate or milk of magnesia are given at bedtime, or morning and night. They act by retarding the absorption of water and so increasing the amount of water that reaches the colon. This prevents too great concentration of the feces in the colon. The larger expulsive doses of saline cathartics should not be used daily for any length of time, for they bring about a "peristaltic rush" for one or two hours only, and accustom the bowel to an entirely abnormal action.

Of the anthracene cathartics, senna, rhubarb, cascara, aloes, aloin and phenolphthalein, little need be said, for they have been prescribed with great benefit by the profession for many years.

At times the use for a few nights of an injection of one pint of warm olive oil to be retained through the night, together with one of the softening agents by mouth, will have a wonderful effect in starting the bowels on a new line of activity. Indeed, a number of the chronically constipated referred to us for advice as to the need of surgery, have apparently completely overcome their chronic stasis by this treatment. Sometimes it is wise to renew the oil injections for about one week in each month.

Having thus reviewed some of the simpler remedies, we might sum up the treatment of chronic intestinal stasis as follows: Regularity of defecation, measures to improve intra-abdominal pressure, measures to increase peristaltic activity, and measures

to increase the bulk and softness of the colon contents. In the average case attention to habits of life and to the amount and kind of food, and the administration of a softening agent or a very mild laxative will be effective in overcoming the stasis, and, therefore, the toxemia. In severe cases, the addition of an oil enema at night may work a marvelous change for the better. In these chronic cases the drastic cathartics should be omitted from use.

If measures such as those spoken of, when carried out thoroughly, do not overcome the stasis and the toxemia, the question of surgery should be seriously considered.

CLINICAL EXPERIENCE WITH LIQUID PARAFFIN (LIQUID PETROLATUM)*

A COMPARATIVE INVESTIGATION MADE UNDER THE AUSPICES OF THE COUNCIL ON PHARMACY AND CHEMISTRY OF THE AMERICAN MEDICAL ASSOCIATION

W. A. BASTEDO, M. D.

During the past three or four years, "mineral oil" has come into extensive use in the treatment of constipation. Preparations of the Russian and the American oil, both heavy and light, have appeared on the market, but there have been no satisfactory data on which to base a selection of oil for use. Therefore, in order to obtain reliable clinical information concerning the relative efficiency of the different oils, the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association submitted samples of the oils to various clinicians for testing. The following is a synopsis of the investigation, which I have prepared at the request of the committee.

The collaborators were advised that specimens of the best obtainable light Russian liquid petrolatum, heavy Russian liquid petrolatum, and an American brand of liquid petrolatum would be sent out, but that, to avoid bias, these specimens would be distinguished only by numbers or letters. The oils sent out were (1) a light "Russian" liquid petrolatum having a specific gravity of 0.860 at 20 C., (2) a heavy "Russian" liquid petrolatum having a specific gravity of 0.885 at 20 C., and (3) an "American" liquid petrolatum having a specific gravity of 0.857 at 20 C. and being markedly fluorescent. The collaborators were advised that the reports should furnish information as to size and frequency of dose, the agreeableness to the taste, the effect on the stomach, the

* Reprinted from *The Journal of the American Medical Association,* March 6, 1915.

number and character of the stools, the degree of admixture of the oil with the other ingredients of the stool, the degree of leakage of oil about the anus, and the need of other cathartic measures.

Reports have been received from Drs. L. F. Barker, W. A. Bastedo, J. B. Champion, Henry A. Christian with C. K. Drinker and F. A. Hatch, Alfred Stengel and R. L. Wilbur.

CONCLUSIONS

The conclusions to be drawn from the clinical reports are:

Dosage.—Half an ounce to 3 ounces a day. In the same patient, the same amount of each of the oils was required.

Frequency of Dose.—The same amount daily seemed as efficient when given in one dose as when given in divided doses two or three times a day.

Agreeableness to the Taste.—There is a difference of opinion in this regard. Two reports favored the heavy Russian oil. One report favored the light Russian petrolatum. But the taste of any of the samples was so slight as to be a negligible quantity after the patient had taken the remedy for two or three days.

Stomach Effects.—In about 20 per cent. of the patients, the oil produced a slight degree of nausea or tended to repeat. This is most likely in patients who have gastric stagnation with retarded emptying of the stomach. All the oils acted the same in this regard. Vomiting was reported in two cases.

Number of Stools.—To produce one or two copious stools a day the dose required varied considerably, but there was no difference noted on account of difference in the specific gravity or character of the oils.

Character of Stools.—The stools were soft, usually formed, sometimes mushy, obviously greasy. They had a peculiar odor described as sour. Their consistency varied with the dose, but was the same for the different kinds of oil.

Admixture of Oil with Other Ingredients of Stool.—Generally well mixed, but from time to time a patient would have a stool of free oil. This occurred with all varieties of oil. (It necessitated reduction of the dose, and if then the bowels were not active enough, the administration in addition of cascara, aloin, etc.)

Leakage About the Anus.—A disagreeable feature complained

of by many is that when they take enough of the oil to move the bowels, there is sufficient leakage from the anus to keep the neighboring skin continually in a greasy condition, and sometimes to stain the clothes. That there is any difference in this regard between the oils has not been determined.

In the reports, one clinician noted no differences that were not negligible. Another was slightly in favor of No. 2 (heavy Russian) as regards taste. A third reporter did not make comparative tests. A fourth is slightly in favor of "B" (heavy Russian) as regards taste and general suitability. All of the findings of this investigation are based on hospital cases. A fifth reporter favored No. 1 (light Russian petrolatum). He considered it the most prompt in its effect, the most uniform in results, and the most prone to give a satisfactory admixture of the oil with the other materials. The difference, however, from the other oils was not marked. Another reporter noted no special differences.

SUMMARY

The results of this clinical investigation appear to warrant the conclusion that so far as therapeutic results are concerned the differences in the action of the three varieties of liquid petrolatum, namely, light Russian liquid petrolatum, heavy Russian liquid petrolatum and American liquid petrolatum, are too slight to be of importance. Hence the choice between the lighter and the heavier oils, and between the Russian and the American is an open one, to be determined not by therapeutic differences, but by palatability, dependent on the degree to which the refinement of the oil is carried out. The U. S. Pharmacopeia, the revision of which is now nearing completion, no doubt will furnish standards which will insure a suitable product. From the findings of the foregoing report it would appear that a satisfactory standard might permit the use of either Russian or American oil, if suitably refined so as to be as nearly as possible devoid of odor and taste.

CHLOROMA*

A Report of Two Cases, Together With the Roentgenologic Findings

BY EVERETT W. GOULD, M.D., AND LEON THEODORE LE WALD, M.D.

Although this disease has been described since the beginning of the nineteenth century under the names of chloroma, chlorolymphoma, and green cancer, only a comparatively few cases have been reported, and its apparent rarity seems to make it advisable to record the following two cases which have appeared in the Pediatric Service of St. Luke's Hospital.

The most complete description of the disease is given by Dock and Warthin in their excellent report of a case and review of the literature upon this subject. It seems to be fairly well proved by them that the condition arises from a hyperplasia of the parent cells of the leukocytes, especially in the red marrow of the bones, that it also occurs in any of the leukoblastic tissues of the body, and that we, therefore, find neoplasm-like formations in the spleen, liver, lymphatic glands, and, in fact, in almost all the tissues of the body.

Their analysis of the cases which have been reported would tend to show that it much more frequently occurs in children and particularly in males. One of our cases was a girl three and a half years old; the other a boy, five years old.

The chief clinical features which characterize the disease are an exophthalmos, which is produced by a retrobulbar lymphoma; swellings of the temporal and occipital regions, due to the involvement of the cranial bones; enlargement of the spleen and lymphatic glands throughout the body, especially of the cervical region; and a severe and progressive anemia causing weakness, fever, and emaciation.

* Reprinted from *Archives of Pediatrics*, 1916.

Nearly all cases have shown an involvement of the bones of the skull, and it is this feature which gives rise to some of the most characteristic signs and symptoms of the disease. This hyperplasia undoubtedly begins in the marrow of the cranial bones, but soon involves the periosteum and the dura mater, forces the sutures apart, fills up various foramina of the skull, and by pressure upon nerves which emerge from the brain through these openings may cause blindness, deafness, pain, or other symptoms referable to the nerves involved.

Thus the exophthalmos, which is such a prominent symptom, is undoubtedly due to the growth within the orbit of a mass of leukoblastic tissue which originates in the marrow of the adjacent cranial bones and by rapid proliferation forces its way through the foramina or the periosteum itself and presses upon the optic nerve and bulbus oculi. Heyden states that exophthalmos was the first symptom noted in a majority of the fourteen cases which he reviewed. It was also an early symptom in most of Dock's cases. In some reported cases there has been no exophthalmos.

The involvement of the other cranial bones causes swellings which may be so rapid in development and so soft that they simulate a cystic condition. The pain and swelling in various joints of the body, which have been frequently described and which have led to an early diagnosis of rheumatism in several instances, have been proved by postmortem examination to be due to this same hyperplasia of the marrow cells in the adjacent long bones. In various reports nearly all the bones of the body have been cited as being the seat of this change, and, although postmortem examination was not made in either of our cases, nevertheless the Roentgen ray findings would seem to indicate that the involvement was both general and characteristic.

The blood picture of chloroma varies greatly in the reported cases. The anemia, which is so marked and so progressive, causing early weakness and emaciation, is believed to be due to the rapid proliferation in the bone marrow of the leukoblastic tissues, resulting in the crowding out of the elements from which the red blood cells are derived. The anemia therefore depends upon the deficient production of red cells rather than upon any toxic destruction of them. The hyperplasia of the leukoblastic

elements leads to the frequent appearance in the blood stream of large numbers of white blood cells of various types, and has led to its classification as a form of leukemia. In most of the reported cases this characteristic leukemic picture has been found at some time during the course of the disease, especially during the later stages. The predominating type may be any one of the normal or leukemic types of the leukocytes, depending upon the stage of proliferation; or, as Dock has suggested, the hyperplasia of the parent cells may reach such a stage that the new forms do not reach the blood stream in such numbers as to give the picture of a leukemia. An aleukemic chloroma is therefore possible. During the time that our cases were under observation the blood picture was never that of a leukemia. Frequent examinations were made, but the time during which the blood was carefully watched represented only a small part of the duration of the disease.

The name chloroma was given to the disease on account of the peculiar green color which was frequently seen when the tumors were removed from the body, or seen postmortem. This green color may be found in any of the new growths, or even in a blood clot within the heart, but is a more prominent characteristic of the hyperplasias of the bone marrow. The nature of this coloring matter is still unknown. Observers differ widely in their descriptions of its characteristics. Most of them state that it fades rapidly when exposed to the air, but the color has been seen to grow deeper when exposed. That it is due to broken-down blood pigment is suggested by some. Dock believes that it is probably a parenchymatous color and that it is possible that processes morphologically like chloroma may lack the characteristic green color. Such cases should be classed with the non-chloromatous leukemias.

In regard to the histologic characteristics of this disease some very important observations have recently been made by pathologists to show that it does not belong to the lymphomata, sarcomata, or lymphosarcomata. Rosenblath urged the rejection of its classification with the lymphomata because so many of the tumors, which might, from their green appearance, be considered as such, differ essentially in many of their characteristics. They

Fig. 1—Case 1. S. R., Female, aged 3½ years. Note exophthalmos

have no capsule, but grow rapidly and diffusely through muscles, fat and bone.

Guembel emphasizes the difference between chloroma and sarcoma. He points out the fact that it is impossible to find the primary growth, that there is an absence of giant cells, that there is no bone formation, and that circumscribed metastases in internal organs are wanting.

That the tumors of chloroma are composed largely of cells of leukoblastic origin was perhaps first observed by Klein and Steinhaus, who found that neutrophile cells, especially myelocytes, constituted a large part of the tumor mass. Large and small lymphocytes with eosinophile granules were frequently predominant, and they, therefore, classified the disease with the leukemias.

The thorough microscopic examination of Warthin would show that in his case at least the tumors which involved the vertebræ and the prevertebral tissues were primary in the bone marrow spaces, that they infiltrate or grow through the lymphatics and blood-vessels or break through the bone and secondarily involve the periosteum and surrounding tissues. Section shows that the normal marrow tissues are almost entirely replaced by cells of the large lymphocyte type, most of them showing eosinophile granulation. Transition forms of various kinds are numerous, but the whole vertebral tumor is made up principally of cells of leukoblastic origin with a scanty and delicate tissue reticulum, and with little evidence of new blood-vessel formation. Nodules of similar histologic structure were found in various organs of the body arising from intravascular proliferation and seeming to have little influence upon the surrounding tissues except by pressure and atrophy. There is little manifestation by the organ involved of a reaction in the nature of a resisting proliferation of the tissue cells, or the development of a reticulum and blood-vessels.

The Roentgen ray findings in the two cases of chloroma studied appear to be fairly characteristic, especially in regard to the proliferation of bone tissue in the skull producing a separation of the suture lines. (Figs. 3 and 5.)

In the second case studied there are characteristic changes in the long bones. The process appears to be essentially a rarefac-

tion and proliferation in the medullary cavities over a variable distance from the surface of the shaft. (Figs. 6 and 7.) The absence of epiphyseal changes distinguishes the condition from infantile scorbutus and rachitis. The combination of the periosteal changes with the changes in the medullary cavity distinguishes the condition from a myelomatous lesion. The extent of the process distinguishes the condition from sarcoma. The changes in the medullary portion of the bone appear to be distinctly different from syphilis.

Our findings are exactly in accord with the gross pathologic changes described by Warthin. As far as we have been able to ascertain these are the first cases in which the Roentgen ray findings have been recorded.

Case No. 92,962. S. R., girl, three and one-half years. Admitted to hospital January 27, 1913. American, born of French parents. Family history negative. Was a full-term child, normal labor, nursed nine months. Had pertussis eight months before admission. Two months later had pain in right side of abdomen and chest. No pathologic cause determined at Babies' Hospital, where patient was treated at that time.

Present illness followed closely upon the inception of the above pain and was characterized first by weakness and difficulty in walking. Dr. Lewis S. Aronson, who saw the child, states that the chief complaint at that time was weakness of lower extremities and edema of upper eyelids. There was no exophthalmos, no edema or swellings of the scalp, and the inguinal glands were the only ones enlarged. The child has been unable to walk for four months. Swelling of the face and neck and bulging of eyes began about six weeks ago. Has recently had slight cough.

Chief Complaint—Progressive pallor and weakness, swelling of neck, and protruding eyeballs.

Physical Examination—A well-developed and well-nourished child, anemic and slightly icteroid. Mentality and special senses do not seem impaired. Circumference of head 21 inches. Scalp edematous and tender. Beginning at root of nose and extending fan-shaped upwards over forehead is an edematous area 6 cm. wide at its base. Temporal veins distended. Face puffy, especially on left side. Pupils widely dilated, react very slightly; marked exophthalmos, both eyes. Ears show no discharge, no

Fig. 2—Case 1. S. R., Female, aged 3½ years. Note the enlarged glands in neck; bulging in temporal region; and exophthalmos

mastoid tenderness. Drums are lusterless, landmarks poorly seen, but there is no bulging. Tongue slightly coated, twenty teeth, upper incisors decayed. Tonsils hypertrophied. On left side of neck the anterior cervical glands are greatly enlarged, several large nodes being matted together. Over these are distended veins. There are also several enlarged glands in postcervical region and a few on right side of neck. Some of these glands seem tender. There is no rigidity of the neck. Axillary and inguinal glands moderately enlarged.

Lungs negative, except for slight dullness at left base posteriorly where breath sounds are higher pitched.

Heart is normal in size, no murmurs. Action rapid, regular in rate and rhythm.

Spleen percusses large, but is not palpable. Liver not palpable. Knee-jerks exaggerated. No Babinsky. No Kernig.

CLINICAL NOTES AND FINDINGS

The temperature ranged from 99 to 102.4 F., usually around 100 F., and the pulse from 120 to 140.

January 28th. Red blood cells, 2,760,000; hemoglobin, 40 per cent.; white blood cells, 9,300; polynuclears, 63 per cent.; lymphocytes, 37 per cent. Red cells stain faintly, central depression marked, no unusual or nucleated forms observed. Vaginal smear negative for gonococci. Nose and throat cultures negative for diphtheria. Urine: Turbid, neutral, 1,025, no albumin, no sugar. Wassermann and von Pirquet tests negative.

January 31st. Eyes examined by Dr. Colman W. Cutler. *Left:* Retina has granular grayish look with few minute white dots scattered here and there. Vessels poorly filled. Nerve sharp and pale. *Right:* Retina same as in left. Appears unhealthy—anemic. Probably a trophic condition. Limitations of motility in all directions. Exophthalmos probably due to enlargement in the orbit behind the eye.

January 31st. Gland removed from right inguinal region. It was soft and friable, and contained white areas softer than the rest of the node.

February 3d. Child is worse; stuporous; vision and hearing seem impaired. Eyes examined by Dr. Cutler: Discs are a little paler. Retinæ unchanged. Loss of vision probably due to pressure on nerve.

February 4th. Exophthalmos increasing. Edema of forehead more extensive. White blood cells, 7,400; polynuclears, 63 per cent.; lymphocytes, 37 per cent.

February 5th. Vision apparently lost. Nerves still pale as before, but outlines definite. Enlarged glands in left side of neck excised by Dr. John Douglas. Specimen consists of three pieces, about 1 cm. in diameter, from cervical glands. On section they show small bright yellow areas.

Microscopic examination by Dr. F. C. Wood: The general picture of the sections is that of the marrow of cancellous bone in which the cells have been crowded together leaving out the red cell element. The normal lymphoid elements of the node are almost completely replaced by these cells, which resemble marrow cells. The largest portion of the cells is made up of mononuclears, which have a faintly staining protoplasm and a large, clear oval nucleus with a fine chromatin network. There are comparatively few mononuclears of the lymphatic type. Scattered through the sections are great numbers of giant cells of both the mononuclear and the polynuclear types. Some of the mononuclears have a large, round, faintly staining nucleus that nearly fills the cell and has a definite chromatin network. Others have a nucleus that takes an intense stain, is irregular in shape, and shows no internal structure. The polynuclear giant cells have from two to ten or more nuclei. These appear to be in all respects similar to the megakaryocytes of the bone marrow. The larger number show distinctly separate nuclei, but in a few the nuclei seem to have an incomplete segmentation. Eosinophiles are easily found. These are mainly of the myelocytic type, but a few have larger cell bodies and appear to have two or three nuclei. Mitotic figures are occasionally seen in the smaller mononuclear cells. There is a type of mononuclear cells having a very deeply pyknotic nucleus, which is spindle-shaped and nearly fills the cell. These cells appear to be most abundant where the connective tissue is most dense. Many of the blood vessels are blocked by cells of this same type. The connective tissue framework is considerably in excess of that in a normal node. The microscopic examination of the node excised from the inguinal region discloses an appearance similar to that of the cervical nodes, except for the presence of a greater amount of connective tissue. *Diagnosis:* Chloroma.

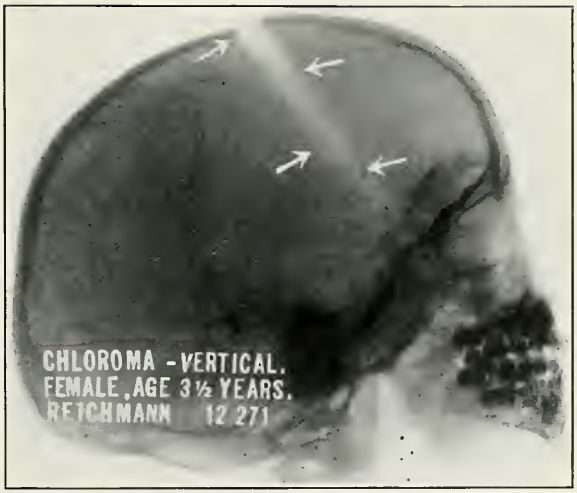


Fig. 3.—Case 1. S. R. Note the marked separation of the coronal suture

February 6th. Urine negative. Bence-Jones albumose not present.

February 8th. Exophthalmos slightly diminished. Discs and retina same as before.

February 9th. Lumbar puncture: 8 c.c. of clear fluid removed, slightly increased pressure. Cells: 2 per c.mm., lymphocytes, 100 per cent.

February 10th. Red blood cells, 1,870,000; hemoglobin, 30 per cent. Several normoblasts found. White blood cells, 16,100; polynuclears, 50 per cent; lymphocytes, 50 per cent. Abdomen distended, no fluid.

February 11th. Swellings appearing in temporal and occipital regions of scalp. Knee-jerks active. No clonus. No changes in eye grounds.

February 12th. Child unconscious. Swellings on scalp seem cystic. No fluid obtained on aspiration.

February 13th. Circumference of head 22 inches. Child died. Postmortem examination not allowed.

Radiograph made January 29, 1913, showed marked enlargement of the mediastinal lymph nodes with slight displacement of the trachea to the right.

February 1, 1913. Anterior radiograph of the chest confirmed the findings of enlargement of the mediastinal lymph nodes.

February 5, 1913. Examination of the skull showed a most striking condition. There was a distinct separation of the coronal suture, producing a wedge-shaped gap measuring 1 cm. at the vault. (Fig. 3.)

February 12, 1913. Radiographic examination of the head confirmed the previous findings of separation of the coronal suture and also showed a separation of the parietal sutures.

Stereoscopic examination confirmed the finding of separation of the suture lines of the skull, including the occipito-parietal regions.

Case No. 96,849. F. P., boy five years old. Admitted to hospital October 21, 1913. American, born of Italian parents. Four other children alive and well. Mother has had three miscarriages. Full term child, normal labor, nursed nine months, teeth at ten months. Walked at fourteen months. Always large and healthy.

Parents date present illness from vaccination seven months ago. A few days subsequent to this the child developed fever and this was followed by a stiff neck and later by tenderness of right knee, left knee, arms and shoulders. Five months ago swellings appeared on head most prominently behind the right ear, but these are said to have temporarily subsided in a few days as result of use of ice bag. At the time that the swellings appeared the eyes also began to protrude, and, although they have seemed to recede at times they have become much more prominent during the past month. Was told by a physician four months ago that the heart was affected. Feet and ankles swollen for one month and abdomen has been becoming more distended during that time. Vision and hearing have seemed normal. Has become very irritable of late.

Chief Complaint—Exophthalmos, swellings on head, and enlargement of abdomen.

Physical Examination—Patient is a poorly-nourished boy of five years who appears chronically ill; is emaciated and has yellowish hue to skin. Head is very irregular in shape, due to subcutaneous tumors. The largest, over the right parietal eminence, is about 6 cm. in width. All are attached to the periosteum and are fluctuating and slightly tender. All are within the region of the hairy scalp. The head measures 53.5 cm. in circumference. Ears show no discharge or mastoid tenderness.

Eyes are prominent, showing distinct exophthalmos. The lids are slightly edematous. Pupils are equal and react to light and accommodation.

Nose and mouth are normal, except for a few decayed teeth.

Neck shows no stiffness. There are masses of enlarged nodes on either side of the neck, in submaxillary regions and both in front of and behind the sternomastoid.

Lungs: There is some dullness at both bases posteriorly, where many fine, moist, subcrepitant râles may be heard at the end of inspiration.

Heart: Apex is 7 cm. to left of midsternal line in fourth space. Right border percusses 3 cm. to the right of midsternal line. There is a rapid diffuse impulse but no thrill. There is a loud systolic murmur heard all over precordium, most marked near the sternum and transmitted to axilla. A short presystolic murmur

Fig. 4—Case 2. F. P., Male, aged 5 years. Note exophthalmos; bulging of abdomen; swelling of temporal regions of skull

is heard just inside the nipple. Pulses are equal, soft, and rapid and of fair force. There is a marked suprasternal pulsation.

Abdomen is very prominent and is 64 cm. in circumference. Liver edge palpable at level of iliac crest and crosses midline at level of umbilicus. Spleen is also palpable below level of umbilicus.

Few enlarged nodes in axilla and groins. Genitals normal. Knee-jerks present. No Babinski. Slight edema of feet. Tibiæ tender.

CLINICAL NOTES AND FINDINGS

The temperature of the patient varied only slightly during his stay at the hospital, ranging from 100 to 101.2 F. The pulse range from 120 to 140, and respirations from 40 to 50.

October 21st. Red blood cells, 2,300,000; hemoglobin, 25 per cent.; white blood cells, 13,500; polynuclears, 72 per cent.; lymphocytes, 28 per cent. Marked central depression of cells, few normoblasts, aniso- and poikilocytosis. Urine: Neutral, turbid, 1,030, no albumin, no sugar, diazo negative, no Bence-Jones albumose, amorphous sediment.

October 22d. Nose and throat cultures negative for diphtheria. von Pirquet negative. Wassermann negative.

October 23d. White blood cells, 11,000; polynuclears, 60 per cent.; lymphocytes, 38 per cent.; eosinophiles, 2 per cent. Urine negative.

October 25th. Red blood cells, 2,400,000; hemoglobin, 25 per cent.

Eyes examined by Dr. Colman W. Cutler. No evidence of increased intracranial pressure. Right disc pale. Pigmented area, probably congenital.

October 27th. Two lymph nodes excised from right side of neck. The larger one is friable and apparently contains caseous material. The smaller one is firm and smooth.

October 29th. Red blood cells, 2,100,000; hemoglobin, 25 per cent.; white blood cells, 16,000; polynuclears, 64 per cent.; lymphocytes, 36 per cent. Urine negative—Bence-Jones albumose not found. The patient is getting progressively weaker.

October 31st. Swelling over right parietal region softer and almost cystic to touch. Exophthalmos not increased. Liver and spleen remain about the same size.

November 2d. Red blood cells, 3,100,000; hemoglobin, 30 per cent.; white blood cells, 16,500; polynuclears, 64 per cent.: lymphocytes, 36 per cent.

November 5th. Physical signs in general, unchanged. Swellings on skull increasing in prominence. Lower extremities edematous. White blood cells, 12,500; polynuclears, 72 per cent.: lymphocytes, 28 per cent. Slight epistaxis.

November 7th. Red blood cells, 2,300,000; hemoglobin, 28 per cent.

November 11th. Exophthalmos apparently diminished. White blood cells, 17,000; polynuclears, 68 per cent.; lymphocytes, 32 per cent. Eight normoblasts found in counting 100 white blood cells. Urine normal. Slight epistaxis.

November 13th. General condition failing. Upper lid of left eye discolored and edematous, apparently due to infiltration from tumor mass above. Purulent discharge from conjunctiva. White blood cells, 13,500; polynuclears, 66 per cent.; lymphocytes, 34 per cent.

November 14th. Lymph nodes excised from left side of neck. Abdomen measures 67 cm. in circumference. General condition the same. Voice much fainter. White blood cells, 24,000; polynuclears, 78 per cent.; lymphocytes, 22 per cent. Discharged against advice. Patient became progressively weaker, and died about ten days after leaving the hospital. Postmortem examination not allowed.

Report on examination of lymph nodes by Dr. F. C. Wood:

Macroscopic: Specimen consists of single gland from neck measuring 1.5 x 1.2 x 0.8 cm. The capsule is thin and gland appears congested. On section the tissue has yellowish tinge with small hemorrhagic areas. There are a few light areas suggesting necrosis. In general, the tissue is pale and cellular. A small gland attached to the capsule appears hemorrhagic.

Microscopic: In the peripheral lymph sinuses there is a collection of round cells, slightly larger and less deeply stained than the lymphoid cells. They lie in a delicate reticulum and there is considerable blood about them. In some portions of the gland these have displaced the lymphoid structures, which appear as deeply staining islands. Some portions of the structure are markedly hemorrhagic. *Diagnosis:* Chloroma.

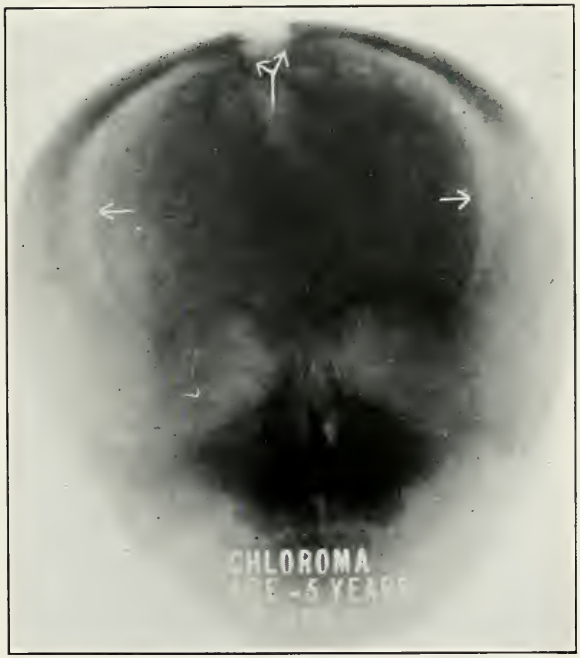


Fig. 5.—Case 2. F. P. Note the wide separation of the suture lines of the skull, especially the coronal and frontal regions

Radiographic examination made October 22, 1913, showed a most striking condition of the skull; there was a distinct separation of the suture lines, especially marked in the region of the coronal suture and the parietal sutures. The separation of the frontal suture measured about 2 cm. at the vertex. (See Fig. 5.) The condition is similar to that previously noted in a case of chloroma and this appears to warrant the probable diagnosis of this condition when taken in conjunction with the findings in the other bones.

Examination of the chest showed an extensive involvement of the mediastinal lymph nodes.

Examination of the upper abdomen showed the diaphragm pushed upward on both sides, indicating a distinct enlargement of the liver and of the spleen.

The bones of the upper extremities showed marked changes of a very peculiar type. The medullary portion of the long bones showed a peculiar mottling due to rarefied areas. The periosteum was distinctly raised from the surface of the bone over the greater portion of the shaft. The epiphyseal region showed comparatively little departure from normal, except for changes above noted. The peculiar combined involvement of the medullary cavity and the periosteum has not been observed in other bone lesions, and as the gross and pathologic findings agree with those found in chloroma it appears sufficient to warrant a diagnosis of chloroma when taken in conjunction with the history of the case. (Figs. 6 and 7.)

Examination of the lower extremities showed an involvement of the long bones exactly in the same manner as observed in the upper extremities, that is to say, there is a periosteal change combined with a medullary change.

Inasmuch as a postmortem examination was not allowed in either of these cases, and the blood picture was never found to be that of a leukemia, the diagnosis might perhaps be questioned. The clinical history and findings were so characteristic, however, and the *x*-ray examinations so confirmatory that it would seem difficult to arrive at any other conclusion. As has been pointed out by several investigators, the blood picture varies greatly in the reported cases and also in the same case on different examinations. The peripheral circulation may not contain the atypical

cells of leukoblastic origin which predominate in the tumor masses. An aleukemic chloroma has even been suggested. The fact that our cases did not give the picture of a leukemia during the time they were under observation would not seem, therefore, to invalidate the diagnosis.

The glands which were examined in our cases did not present the characteristic green color which originally gave the name to the disease. This fact, again, does not seem to be any argument against the diagnosis, for the green color is much more characteristic of the tumors involving the bones and adjacent structures and we have no reason to doubt that these deeper tumors would have manifested the green color in our cases.

The color is no longer considered an essential feature of the disease, but it would perhaps be better to class a case which was thoroughly examined and did not show the green color as a non-chloromatous leukemia.

Our present knowledge of the disease leads to the conclusions which were recorded by Dock and Warthin, that

(1) Chloroma is a tumor-like hyperplasia of the parent cell of the leukocytes, primary in the bone marrow, the periosteum being involved only secondarily.

(2) As a result of this leukoblastic hyperplasia atypical leukocytes or leukocytes corresponding to some of the normal types may appear in the blood stream in varying numbers.

(3) The essential difference between chloroma and other forms of leukemia is the marked neoplastic character of the former and the formation of the green infiltrations and metastases.

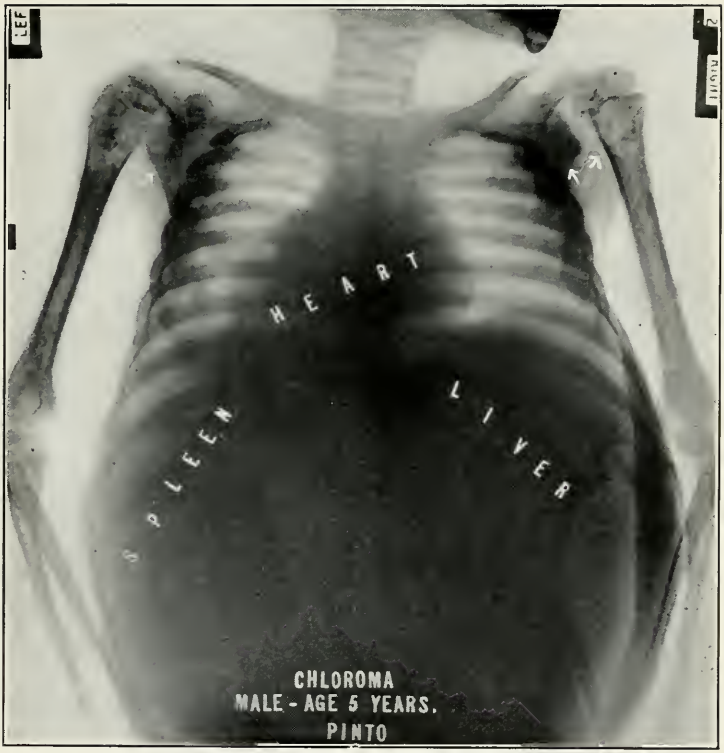


Fig. 6—Case 2. F. P. Note the marked changes in the medulla of the bones about the shoulder joint

Fig. 7—Case 2. F. P. Note the periosteal changes combined with the medullary changes especially in the long bones

REPORT OF PEDIATRIC CLINIC

It has been thought that a brief résumé of the work of the Pediatric Clinic since its occupancy of quarters in the Travers Pavilion in October, 1911, may be of interest.

The growth of this Out-Patient service may be said to have begun at that time, but, unfortunately, the records of the daily attendance were not kept separate from those of the general Medical Clinic for the first six months thereafter, and so no figures are available for comparison. The attendance for the year ending September 30, 1915, however, was 6,906, as compared with 5,183 for the year ending September 30, 1913. This gives an increase of 1,723 for the two years. The monthly average increased from 431 to 719, and the daily average from 17 to 23 patients.

The general efficiency has been greatly improved by the services of an attending nurse, in addition to the supervising nurse. This has been brought about by an arrangement whereby certain senior nurses of the Training School are given three months' service in the Children's Clinic in connection with Social Service work. The work of the nurse consists in taking temperatures, weights, and measurements of patients, and otherwise preparing them for the physical examination. Much time is thus saved the physician and more thorough examination can be made.

A visible card index has been installed and by its aid important cases are checked up and kept under observation, so that if the patient does not return on the day specified, the nurse can investigate either by postal or personal call, and urge that the patient return for further observation and treatment.

If the patient is ill enough for hospital care and for any reason it is impossible to admit him at the time, he is seen at his home by the nurse on the following day; if found then to be still sick enough to go to the hospital, another attempt is made to have him admitted; should this still be impossible, he is looked after by the

physician or nurse at his home until such admission is secured or the case is ended. The physicians hold themselves in readiness to respond to these emergency calls and considerable good work has been done in thus following up our cases and keeping them within our own circumference of observation.

The physicians in attendance at the Children's Clinic also enjoy the privilege of following their cases after admission to the wards and for some time past they have made rounds with the attendings one day in each week. Exchange slips are used, both in transferring patients from the clinic to the wards and when the patient is discharged from the ward and returned to the clinic. These contain a synopsis of the history, the clinical findings, and the treatment of the patient for the guidance of the physicians in the department to which the patient is transferred.

The establishment of a clinic laboratory has been of great assistance to the physicians in the Out-Patient Department. Urinalyses, blood counts, examinations of nose and throat secretions. and vaginal smears are here made. Patients requiring the Wassermann test are sent to the Pathological Department of the Hospital. These aids to a clearer understanding of our cases with the prompt and willing service of the Roentgen-Ray Department and the immediate co-operation of any of the special clinics should serve to curtail effectually any tendency to careless methods of diagnosis.

Our clinical cases cover practically the entire range of pediatrics. Feeding cases and the gastro-intestinal disorders in general of infancy and childhood are the conditions most frequently presented. Many cardiac cases are also seen; these we attempt to keep under the closest observation, as we do also the cases of chorea, tonsillitis, and other expressions of the rheumatic diathesis. In connection with the choreic patients, the co-operation of the Social Service staff has been most helpful in finding places for those who are convalescent. Many patients have been sent to Chappaqua, in Westchester county, to Holiday Farm, at New Hamburg, both under the auspices of the Children's Aid Society, and to the Boys' Pavilion of the Burke Foundation, the latter place admitting mild cardiac cases alone. It has been extremely satisfactory to note the great improvement in most of the cases that have had the advantage of a few weeks' residence in these homes.

We see large numbers of cases of contagious diseases, measles and whooping cough being perhaps the most common. Great care is exercised to prevent these patients from mingling with others on the admission line. Those accompanying the children are questioned by the admitting clerk relative to the presence of sore throat, skin rashes, or suspicious cough, and if a contagious diseases is suspected the child is immediately placed in the isolation room at the entrance to the Clinic. The patient is here seen at once by a physician and if the contagious nature of the ailment is confirmed, he is held for the Health Department's care, or treated and returned to his home, provided the case is ambulatory and the home within easy walking distance. The cases returned to their homes are followed up, and if they cannot be cared for by their family physician they are sent to a suitable hospital or looked after by our own physicians and nurses.

The pertussis cases are excluded absolutely from the general reception room of the Clinic and examined and treated in the isolation room. There is a distinct need for more hospital accommodation for the cases of pertussis with complications like those of a pneumonia or a meningitis. The City Hospital and the Willard Parker are the only places where they are admitted, and these accommodations are limited. The matter of a special clinic for pertussis cases at St. Luke's was seriously considered some time ago, but it was found that the requirements were so exacting as to prohibit the undertaking for the present.

A pertussis vaccine has been used therapeutically within the last few months in a small number of cases in Division A of the Pediatric Clinic with results as follows: Of 19 cases thus treated there were definitely improved 13; improved after the third injection, though seen very late in the disease, 1: unimproved or very doubtful, 5. Of those considered improved the testimony was invariably that of a marked diminution in the number of spasms in twenty-four hours and especially a lessening of the vomiting. The longest duration of the disease as regards the whooping was 42 days; the shortest 14 days; 2 cases were 21 days each; 1 case each 16, 20, 23, 25, 26, 28, 30, 33 and 35 days. This gives an average duration of the whooping of about 26 days. Pertussis combined vaccine was used, 1 c.c. being the amount given at each

injection. The initial dose, designated No. 1, was of 150 million bacteria composed of

25 million B. pertussis (Bordet)
25 million B. influenzæ
25 million pneumococci
25 million streptococci
50 million staphylococci (aureus and albus)

The succeeding doses were of Nos. 2, 3, and 4, containing respectively, 300, 600 and 1200 million bacteria per c.c. The injections were given in most cases two to three days apart; a few at from five to seven day intervals.

The number of injections given was as follows: in 1 case, 9; in 3 cases, 3; in 3 cases, 6; in 2 cases, 5; in 4 cases, 4. No marked reactions following the injections, such as a rise of temperature or prostration, were noted in any of the cases. There was a slight reaction in one or two.

Apropos of this method of treatment, it may be remarked that the Department of Health of this city recently made a statement to the effect that very good results had been obtained at the city clinic by the use of a pertussis vaccine prepared at their own laboratories. The cases were said to have been modified in their intensity and the course decidedly shortened.

WILLIAM C. CALHOUN, M.D.
CHARLES HENDEE SMITH, M.D.

THE BONE GRAFT IN TUBERCULOUS DISEASE OF THE SPINE

T. HALSTED MYERS, M.D.

My early orthopedic training was along strictly conservative lines, and by these methods I have secured a number of very perfect cures of Pott's disease in each region of the spine. These were obtained only in those cases where the entire cooperation of the patient and his family was given, and by long continued exacting attention to all the details of mechanical treatment.

When these surgical methods were introduced, which promised to produce much more quickly a bony ankylosis of the diseased vertebræ, thereby put the parts in a more favorable condition for cure, and also prevent the increase of the existing deformity, I decided to adopt them in a certain number of cases where satisfactory results by mechanical treatment seemed improbable.

As I believe statistics alone are very often misleading, the following cases are recorded in sufficient detail to enable those interested to judge for themselves the effects of the treatment.

The Bone Graft

It is interesting to determine the length of time the graft remains visible by the *x*-ray. In four cases it was not possible to get *x*-ray pictures months after the operation. In three cases only, the graft could not be distinguished: In case 8, a child, 6 years old, the graft, covered with periosteum, had disappeared at the end of ten months. In case 2, periosteum not retained, child, 5 years old, it could not be seen at the end of a year. In case 15, a man, 28 years old, it could not be seen twenty-five months after operation.

In case 9, child, 2½ years old; and case 8, a boy, of 6 years, only a trace of the graft could be demonstrated at the end of six and ten months respectively.

On the other hand, the graft showed clearly in case 6, man, 21

years old, at thirteen months; in case 12, man, 35 years old, at twenty-five months; and in case 4, woman, 27 years old, at three years after operation. The other cases were intermediate in duration.

These observations seem to indicate that the grafts in adults last longer than those in children.

In some cases, as 2 and 15, the vertebræ, seemed to be fused where the grafts were inserted, though the grafts themselves could not be seen. When bone chips were inserted by the side of the graft, in one case one punctured the skin and was removed; and in the other cases a chip remained unattached and more or less painful for months. They do not seem necessary, and I am not inclined to use them again.

In one case, the graft was exposed to reduce angulation; it was found to be firmly joined to the spine by bone, and bled when cut, showing circulation in it seven months after operation.

In the case in which the patient died five months after operation, the graft was found firmly united to spinous processes. This was a case of multiple osteomyelitis; and when the graft was cut from the tibia, although there had been no symptom of trouble in this bone, a little serous effusion was seen under the periosteum at one point, and this part of the bone was not used. It would, therefore, seem wise in cases with multiple foci to take before operation an *x*-ray picture of the tibia to be used. This was the only case in my series in which there was any infection of the tibia after operation, and the occurrence of infection was probably due to the condition of the bone at the time of operation. In the others cases healing was prompt, and the bone removed was reproduced very quickly whether the periosteum had been removed with the graft or not.

The use of the Esmarch bandage on the leg prevents some loss of blood, which may be a matter of importance in a weak patient; enables one to see the field of operation better, and, as Dr. Ryerson has suggested from his experience, reduces the chance of fatty embolism.

In case 13, the Esmarch was applied above the knee, while the interference with circulation occurred at a point six inches below the knee, at the site of the local postoperative hemorrhage.

Abscess

Every abscess which had been known to exist is shown in the *x*-ray pictures.

In case 7, there is a fusiform shadow at the sides of the vertebræ from the 9th to the 11th dorsal. There is no other symptom of abscess. The deformity has increased in this case also, and there has been some radiating pain at site of disease. No other sign suggesting abscess.

In case 11, there is a very regular shadow from the 5th dorsal to the 1st lumbar. This patient has had more feeling of weakness than usual, and some radiating pain above lesion in vertebræ but within limits of the abscess. No other symptom of abscess.

In case 12, there is a fusiform opaque mass on each side of the vertebræ from the 9th dorsal to the 2nd lumbar. No other symptom suggests abscess.

In case 9, abscess appeared under the skin six months after operation. No fusiform swelling about vertebræ, but increased opacity external to spine at site of swelling.

In case 5, multiple osteomyelitis, the operation had no effect on the amount of discharge.

In case 13, also multiple osteomyelitis, the amount of discharge has decreased at least one half.

In case 14, after aspirations, the pus collecting has become much thinner and less in amount.

The Effect of the Operation on the Paraplegia

I have made a study of a very large number of cases of Pott's diseases with paraplegic symptoms[1] to determine the probable duration of these symptoms in cases treated with braces and rest in bed. These cases in the mid dorsal region were paralyzed on the average nine and one-half months.

In case 1 of this series, the patient could walk in four months, though the exaggeration of knee jerks did not disappear for over a year. In case 4, the patient was walking in five months, while the average duration in my former cases was eight months. In case 11, the patient had no symptoms after three months, as compared with an average of six months. In case 12, the patient was paraplegic in February, 1913; and in November, 1913, when the

operation was done was still paraplegic, but was able to walk in March 1914, four months later. The average for this location was eight months. The operation has, therefore, seemed to shorten the period of paraplegia in all the cases I have seen.

In weighing the results of this operation, we must consider how much improvement we might expect in the same time from rest in bed, with protection of the spine by braces or jackets.

I view the operation as simply a mechanical device to secure more complete immobilization of the diseased area than we have before been able to secure. This immobilization is not complete, as the articular surfaces and bodies of the vertebræ are not held, only the spinous processes and the laminæ to some extent, are fused. It, therefore, seems to me evident that lateral support also should be continued for a long time after the operation until the adjoining tuberculous surfaces may consolidate. To this lack of postoperative support, I think, are due most of the cases of relapse which are being observed.

Of the sixteen cases here reported, one patient died from status lymphaticus, one from the exhaustion of abscesses connected with multiple foci of osteomyelitis; the operation giving no relief: three are probably better than they would have been without operation; and eleven were cured or very much better than they would have been without operation.

The operation is often indicated in fractures of the spine without symptoms of injury to the spinal cord (cases with such injury are not here considered). Many cases of this kind first develop deformity weeks or even months after the accident, when the fracture is supposed to be consolidated, and the patient is allowed up. This delayed union is due to the small size of the fractured parts which makes it impossible to immobilize them. These weakened vertebræ are especially favorable sites for tuberculous infection.

I think the operation will appreciably shorten the period of Pott's disease, and diminish the amount of existing and probable deformity at very little operative risk.

Case 1. C. W., now 13 years old.

At five years developed malposition, weakness, pain. Six months later kyphosis at 7th dorsal noted. Spinal brace with chin piece applied then, but kyphosis seemed to be increasing

slightly after six months and child was put to bed and kept there two years when she was in fine condition. Then allowed up with spinal brace and chin piece. No pain, no grunting respiration, no increase of reflexes at knee or ankle, no anesthesia, no increase in kyphosis, but ankles and all walking muscles weak. In four months walked about as normal child. A year later developed measles; and a few months later, typhoid fever, and there was a slight increase of kyphosis. Six months after this, all symptoms having disappeared, the chin piece was omitted *gradually.*

June, 1913, (18 months after taking off chin piece), the child was considered cured, but to prevent secondary curves enlarging as she grew the lower part of spinal brace was retained. Weight, 74 lbs., looked well, no cough, no temperature, very active, kyphosis very inconspicuous, height, 4 ft. 5¾ in. In the fall of this year her school mistress, in spite of the history, insisted on giving her setting up exercises with the other children, bending head forward to ground, lateral bending, etc. After a month the child began to be weak in legs, the knee jerks became exaggerated and ankle clonus appeared. There was no pain or grunting, but the attitude in standing and walking became constrained and abnormal. General health was good. Paraplegia was beginning again, and the mother could not endure the idea of bed treatment for the long time necessary. On November 4, 1913, I, therefore, did an Albee operation. On February, 1914, the child left the hospital wearing a brace with considerable increase of power in legs since operation.

October, 1914, kyphosis diminished by operation. Walks poorly yet; allowed to go but ¾ of a mile at a time. Knee jerks still somewhat exaggerated, and ankle clonus at times. Very fat; weight 115 lbs., height 4 ft. 9¼ in. Wears light spinal brace with corset over.

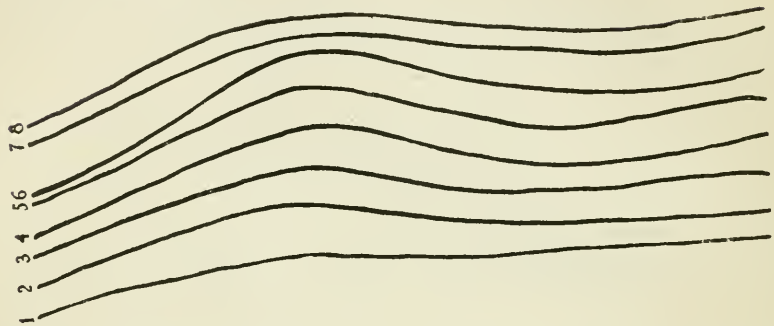
April, 1915. Reported doing very well; gaining in strength and walking better.

January, 1916. Mother reports: walking normally, straight, no pain, no cough. General health excellent.

April 28, 1916. Walking well, no pain, no cough, knee jerks normal, no psoas contraction. General health excellent. Wears ordinary corset.

Case 1.

1. July, 1907.
2. September, 1907. (Put to bed.)
3. March, 1910. Has been walking four months.
4. December, 1911. Following measles and typhoid in summer.
5. June, 1913. Supposed to be cured.
6. October, 1913. Following exercises.
7. October, 1914. A year after operation.
8. April 26, 1916.



Case 2. E. M., 5 years old. Admitted June, 1913. December, 1911, fell down stairs. Physician detected no serious injury.

April, 1913, complained of stiffness of back and pain and weakness of limbs, and spinal deformity was noted.

June, 1913, entered hospital. Kyphosis found from 9th dorsal to 1st lumbar; confirmed by *x*-ray. No knee jerk obtained, limbs stiff. Slight dullness at left base and diminished respiratory sounds at both bases. No râles. Put on Bradford frame with spinal brace also, and kept so till time of operation. December 2, 1913, antitoxin, 800 units, injected.

February, 1914, sudden increase of deformity with tendency to lateral displacement at apex of kyphos.

March, 1914, diphtheria; 3500 units antitoxin injected; rapid recovery.

November 2, 1914, trace of albumin found in urine, sp. gr. 1020, no casts.

November 2, 1914, Albee operation, gas oxygen anesthesia. Median incision from 6th dorsal to 2d lumbar spine; little blood lost. Esmarch to leg; graft 16 x 3 x 1.75 cm., without periosteum, removed. On account of sharp angulation, after shaping graft, had also to nick its concave surface to secure a good fit. Kangaroo tendon retaining sutures.

While applying plaster cast, with child prone, respiration stopped. Cast and chest pressure removed; artificial respiration. In one minute, respiration was fairly good. Applied turtle back of plaster with pelvic and loose thoracic bands and child returned to ward with fairly good pulse and respiration, but unconscious. One hour later spasmodic movements of right hand and arm lasting from five to ten seconds recurred at short intervals, later the left hand and arm were similarly affected. At these times, the jaw was also set, and the legs and feet extended. The pupils were dilated, knee jerks increased, ankle clonus, Kernig and Babinski reflexes present. Child became conscious in nine hours, and spasms of muscles, rapidly diminishing in number and intensity, had entirely ceased within twenty-four hours. The urine was voided in average amount; sp. gr. 1.005, neutral, trace of albumin, many hyaline and granular casts.

November 25, 1914. Condition excellent. Pulse, respiration, temperature, normal. Faint trace of albumin in urine, no casts.

X-ray (Fig. 1) shows graft in place from 7th dorsal to 3d lumbar; seems to be broken at 10th dorsal. Two small calcified glands at root of each lung. Lateral displacement of one-third of an inch to left of 12th dorsal (11th is almost entirely destroyed) under 10th dorsal.

December 2, 1914. Wounds fully healed on first dressing. No psoas spasm, no grunting respiration, slight tenderness over apex of deformity only. Allowed up, and walking. Discharged from hospital.

Case 2.
Operation November 2, 1914.
1. June 24, 1913.
2. October 26, 1914.
3. October 28, 1915.
4. May 29, 1916.

October 28, 1915. Graft does not show; 9th and 10th dorsal are fused and displaced as before over 12th dorsal and 1st lumbar, which are also fused. There seems to be union also between displaced vertebræ.

December 13, 1915. Child at home in excellent condition, without pain.

May 29, 1916. No pain, no psoas resistance. Weight 55 lbs. Condition excellent, no evidence of active disease. Walks strongly and well.

Case 3. M. F., 5 years old. Admitted December 17, 1911.

About six months before this a small swelling was noted in the mid-dorsal region. This constantly increased in size, and just prevous to admission tenderness was present. Weakness of legs had begun some months before admission and now child tottered and had considerable difficulty in walking; was disinclined to stand or walk without assistance. General health fairly good. Heart and lungs about normal. No fever; gastrointestinal condition good. Cervical lymph-nodes enlarged. Swelling of neck noted, but not described. Marked kyphosis in mid-dorsal region (7th and 11th dorsal). Leg muscles atrophied somewhat and power decreased. Knee jerks normal; no ataxia and no sensory disturbance.

He was treated in hospital with spinal brace; developed measles.

December 4, 1912, Albee operation was done. Incision 8th dorsal to 1st lumbar through skin, fascia and periosteum, to tip of spinous processes. Longitudinal section of these spines with Hayes saw to a depth of ½ to ¾ in. rather quickly and easily. Hemorrhage moderate. Incision over crest of left tibia and removal of piece of bone 11 x 2 x 1 cm. No Esmarch was used. Bleeding of soft parts slight; of bone moderate. While the inset was being sutured in place in the groove in spines, the patient ceased breathing, the color remaining very good, and pulse perceptible for some minutes. Caffein and soda benzoate and adrenalin were injected subcutaneously and artificial respiration kept up with oxygen for an hour without effect.

Was this a case of status lymphaticus? Was it surgical shock? The child was in fairly good condition, and the operation had proceeded rather more rapidly than usual. The hemorrhage was not more than moderate.

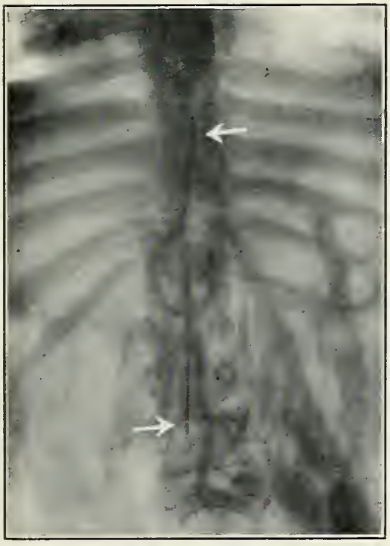


Fig. 1

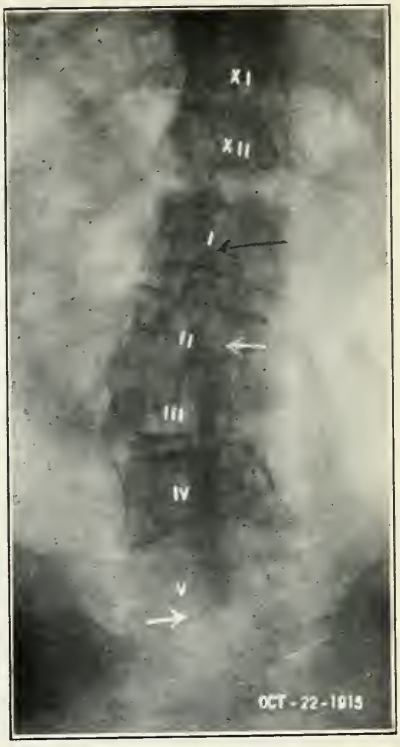


Fig. 2

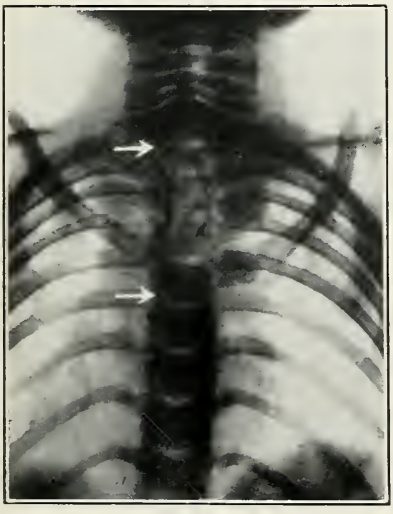


Fig. 3

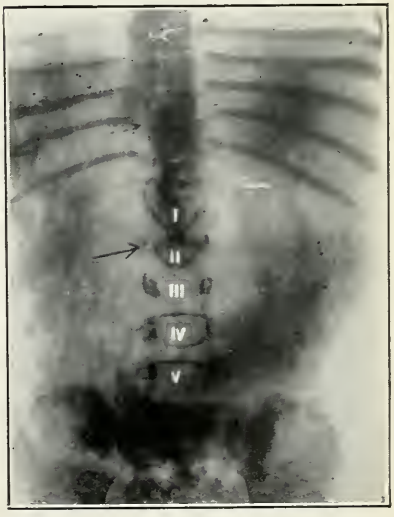


Fig. 4

Case 4. F.S., 27 years old. Admitted July 29, 1912.

Five years ago, abscess appeared to right of 1st lumbar spine. A piece of bone was removed eight months later, and sinus promptly closed. Four years ago, a kyphosis appeared involving 8-10 dorsal spines. Recently a kyphosis appeared at 2d to 3d lumbar vertebræ. Patient holds spine rigidly and complains of radiating pain from lumbar region to abdomen, not definitely enough located to be sure from which focus the pain originates. The woman is neurotic and symptoms given differ from day to day, but there seems to be a great deal of pain, also weakness of spine; there is no paraplegia. There is dullness at both bases and some râles. Wassermann is negative. Von Pirquet is positive. The second kyphosis appeared while she was wearing a brace. She was unable to do anything and had a dependent family. Bed treatment had relieved pain and she was in excellent condition. It was decided to operate on the kyphosis which had recently appeared in the lumbar region.

October 12, 1912, I did an Albee operation. The 1st to 4th lumbar spines were split, and a piece of tibia 14 x 2 x 1 cm. was inserted and held by heavy chromic gut. The periosteum was retained on graft. A plaster jacket was applied over dressings. Wounds healed by primary union.

December 10, 1912. Jacket replaced by a spinal brace; two weeks later patient began to sit up daily, and in two weeks was walking.

February 3, 1913, she had a fall to floor, and anesthesia developed quickly over both gluteal regions, and posterior surface of thighs, legs, and heel. There was also paralysis of vesical and rectal sphincters lasting for nine weeks. The anesthesia very gradually diminished, but did not entirely disappear until twenty-eight months later. The lesion causing these symptoms was in the 3d or 4th lumbar segment of the cord at the 1st level of the 12th dorsal vertebræ, or of the cauda equina at the 4th lumbar vertebræ. That is either at the top or bottom of segment immobilized by the graft. Drs. Bailey and Hunt in consultation both located it at the higher level and advised against operation. Patient was kept in bed for five months, then walked well.

November, 1914. The lumbar deformity is fully corrected; there is diminution of the dorsal. Her general condition is excel-

lent and she does some work at home. There is still considerable pain at times radiating from upper and lower ends of graft.

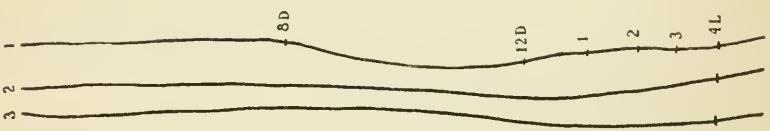
June, 1915. No anesthesia now. X-ray shows complete restoration of tibia. Removable cast worn, and patient exposed to sun three to four hours daily.

October, 1915. Strong dense graft shown extending from 12th dorsal to 4th lumbar (Fig. 2). No psoas spasm. Some pain occasionally at level of iliac crests. Knee jerks normal. Walks about and does work at home.

December, 1915. Re-enforced corset applied in place of plaster jacket.

June 27, 1916. Wears corset still. Sometimes pains about waist. No anesthesia. No psoas contraction, no cough, general health excellent. Severe fall in January, 1916.

Case 4.
Operation, November 12, 1912.
1. January 11, 1913.
3. June 27, 1916.
2. November 1, 1915.



Case 5. J. B., 20 years old. Tuberculous osteitis sternum, lumbar vertebræ, pelvic abscesses, etc.

Following pneumonia two and half years ago, noted stiffness and pain in spine. Four weeks ago jumped from window in a fire. Abscesses then discovered in right and left pelvis. Lumbar spine rigid with well marked kyphosis of 3d, 4th, and 5th lumbar spines. Abscess on sternum opened, and carious bone removed soon after.

December 18, 1914. X-ray pictures showed disease and destruction of 3d and 4th lumber vertebræ. Fifth lumbar and sacrum also affected and change of bony relations in left sacro-iliac joint. Fracture of transverse process of 4th lumbar vertebræ of right side. Von Pirquet positive.

December 18, 1914. Plaster of Paris spica applied and bed treatment. Right pelvic abscess opened spontaneously January

14, 1915, and cast removed. Left pelvic abscess pointing March 2, 1915.

May 13, 1915. Albee operation; ether anesthesia. Curved incision to right of kyphosis. Spinous processes of all lumbar and two upper sacral segments split widely to receive graft. Considerable hemorrhage. Esmarch to leg, and graft, with periosteum retained, taken by motor saw from tibia. This was very thick and fitted accurately in both lumbar and sacral groove. Kangaroo retaining sutures. Long double spica applied, and leg also enclosed in plaster splint. Recovery from operation good. Pelvic abscesses continued to discharge freely. Some infection occurred in leg wound and there was some discharge for months. Sternum sinus continued to discharge. No renal or pulmonary complication.

October 1, 1915. Began to lose strength rapidly, both limbs swelled to hips, and patient died from exhaustion a week later. Graft was found still firmly fixed in position. No further examination was permitted.

Case 6. A. S., 21 years old. Admitted November, 1914.

Patient fell off load of hay two years ago; complained of great pain in groin, was carried home and remained in bed three days. Eighteen months ago, began to have spinal pain, which has been severe for the past six months. There is no history of tuberculosis, pneumonia, pleurisy, or cancer; Wassermann negative; Von Pirquet positive. On admission, there was a well marked kyphosis in lower dorsal region, which had been noted two months. Pain was severe, increased by sneezing, lifting, etc. General condition fairly good. Urine, heart, and lungs normal. Knee jerks absent. Ankle reflex present. Abdomen normal.

September 26, 1914. Gas-oxygen anesthesia. By vertical incision, spinous processes from 7th dorsal to 1st lumbar exposed. Split longitudinally with Hayes saw and chisel. With motor saw the graft, without periosteum, was taken from tibia, and shaped with rongeur to fit groove in spine. Some lateral deviation at apex of deformity made this operation difficult. Some bone chips were laid in along the graft, which was then fastened down strongly in place with kangaroo sutures. Muscles and fascia were united over graft with chromic gut, plain gut for skin; felt padding over dressings, and plaster jacket over this.

Patient suffered a great deal of pain for two days and had to be catheterized four days. Dressed at end of third week. A small bone chip removed from lower end of wound; a small pressure sore at upper end of graft. No pain. Four days later healing complete. Four weeks after operation knee jerks normal. Some pain and edema at upper end of leg wound at end of two weeks. Some serum evacuated. Firmly healed a week later without further pain. Allowed on lawn. Six weeks after operation allowed to sit up partially.

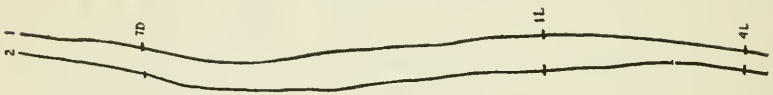
November 14, 1914. Recommended for discharge. Wearing plaster jacket and walking a few steps.

December 16, 1914. Walking well, in good condition, no pain.

April 17, 1915. Plaster cast replaced by spinal brace, and patient allowed to do light work. In excellent condition.

October 27, 1915. Walks freely; looks and feels perfectly well; no pain whatever; no increase of curvature; wears no support. *X*-ray shows graft uniting 7th dorsal to 1st lumbar. Graft is not dense. A lateral plate shows partial destruction and close approximation (fusion?) of 9th and 10th dorsal vertebræ, with angulation at 150°.

Case 6.
1. February 27, 1915.
2. October 27, 1915.



Case 7. M. N., 11 years old. Admitted November 14, 1914.

Patient fell down stairs five years ago, and was taken next day to the Post Graduate Hospital where a plaster jacket was applied. Jackets were worn three years, when she was discharged cured. Appeared well for a year and a half, then, six months ago, pain reappeared in back and mother noted a kyphosis.

She has had a cough for five years and examination shows soms crepitant râles at both bases. *X*-ray shows some calcified glands. Knee jerks are increased. No ankle clonus. Marked kyphosis with apex at 12th dorsal. Some induration in pelvis. Temperature 100°, respiration 24, pulse 24. Von Pirquet positive. *X*-ray shows destruction of 11th and 12th dorsal with fusiform shadow about this region.

December 14, 1914. Spinal assistant brace applied. Temperature soon dropped to normal and general condition improved with bed treatment.

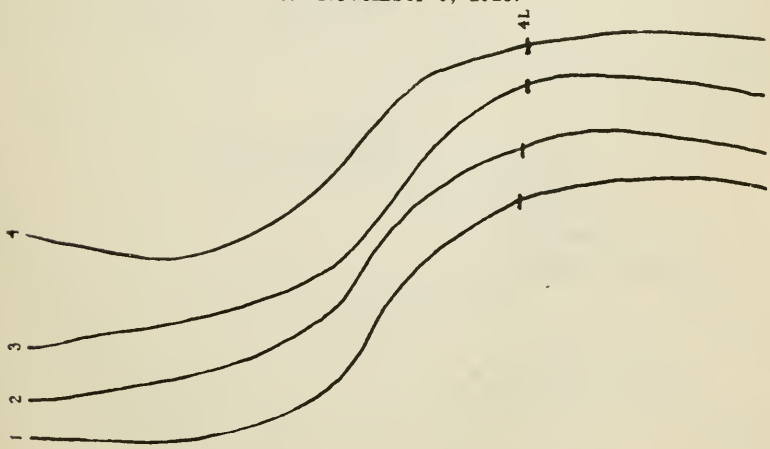
February 18, 1915. Under gas-oxygen anesthesia, the spinous processes from 8th dorsal to 1st lumbar were exposed, split with motor saw and chisel, and the gutter so made packed. Graft was then outlined on left tibia and removed with its periosteum by motor saw and chisel. With rongeur and transverse cuts of saw to make graft somewhat flexible, it was made to fit the groove in spine exactly. It was held in place with kangaroo sutures through interspinous ligaments, and muscles and fascia. Muscles sutured with chromic, and skin with pain gut. Over the dressings the spinal assistant was applied. Plaster cast to leg. Healing by first intention.

May 15, 1915. Leg as strong as ever; no spinal pain; walks carefully, protecting herself; knee jerks normal; no psoas spasm; general health good; urine normal.

June, 1915. Discharged from hospital. Tibia completely filled in.

September 24, 1915. *X*-ray shows graft holding two vertebræ above and two below diseased area. Same opaque area on each side of diseased area.

Case 7.
1. November 6, 1914.
2. February 5, 1915.
3. May 5, 1915.
4. November 9, 1915.



November 9, 1915. Outline shows increase of deformity since walking about. This has occurred above the area fixed by graft. No other change; no pain. Home care very inefficient. Wears brace.

Case 8. K. C., 6 years old. Admitted November, 1910.

Eighteen months before this began to complain of pain in front of chest, and would awake at night crying with pain. Eight months after this a kyphosis was noted and a doctor applied a spinal assistant brace. Not improved by this.

On admission, child was in poor condition; a mouth breather with enlarged tonsils and cervical nodes, contracted chest, and crackling râles at both bases posteriorly. Marked kyphosis in upper dorsal region, apex at 3d and 4th spines.

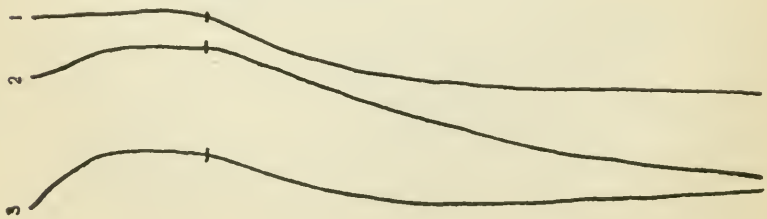
Spinal assistant brace with chin piece applied, and continued until November 15, 1912, when an Albee operation was done. *X*-ray showed the lesion to be in 3d, 4th, and 5th dorsal. The graft, with periosteum retained, was made to fix one spinous process above and one below, the two which projected prominently. A plaster jacket was applied to include the head.

February 26, 1913. *X*-ray (Fig. 3) shows graft in place uniting a confused mass of bone, representing the 1st, 2d, and 3d dorsal vertebræ, as the 1st, 2d, and 3d ribs articulate with it to the 4th, 5th, and 6th dorsal.

March 1, 1913. The jacket was replaced by a spinal assistant brace with chin piece.

July 21, 1913. Was sent to the country. No pain. No exaggeration of knee jerks. Walked with greater freedom and better balance than before the operation.

Case 8.
Operation November 15, 1912.
1. September 10, 1912, before operation.
2. January 28, 1913.
3. January 15, 1914.



September, 1913. *X*-ray shows a faint outline of the graft. This was ten months after it was inserted.

February 4, 1914. General condition good. Walks strongly and well. Still wearing brace with chin piece. The outlines in this case show an increase of deformity, but it is above the area originally diseased, and where the bone appears to be healthy. I have not been able to find this child since February, 1914.

Case 9. M. A., 2½ years old. Admitted January 19, 1915.

A year ago, child, then 18 months old, fell from high chair to floor. Did not appear much hurt at time, but six months later mother noted the kyphos and shortness of breath and a brace was applied. Only within short time has loss of weight been noted. Sleeps well. Von Pirquet test positive. *X*-ray shows 11th and 12th dorsal vertebræ almost completely destroyed. Child seems well nourished, but of low intelligence. Some infiltration about hilus of right lung.

February 4, 1915. Under ether an incision was made from 9th dorsal to 3d lumbar spines; these were split with Hayes saw, the gutter widened with chisel and packed. Under Esmarch control, a graft was removed with chisel from left tibia, periosteum was retained. This was fitted to spinal gutter and held firmly in place with kangaroo tendon sutures passed through interspinous ligaments and muscle fascia. No drainage. Over sterile dressings a spinal assistant brace was applied. Leg protected with plaster splint. Soiled dressings caused two small stitch abscesses which healed promptly.

May 5, 1915. Outline shows considerable decrease in deformity. No exaggeration of knee jerks; no psoas resistance; no pain; no grunting respiration. Habits filthy and cannot be controlled on account of low intelligence.

June 4, 1915. Graft not distinctly visible.

July 3, 1915. Would make no attempt to walk.

August 21, 1915. A large abscess has appeared in right lumbar region; no tenderness or redness.

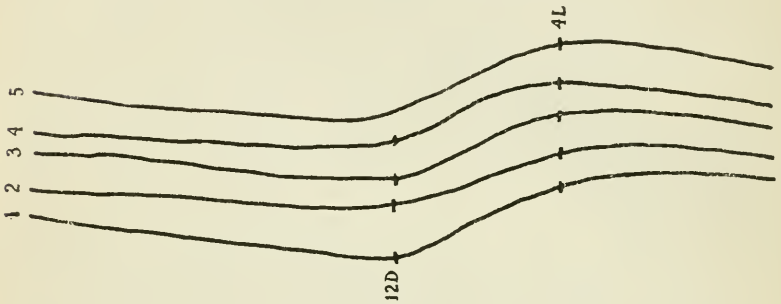
September 11, 1915. The interspaces between 11th dorsal and 3d lumbar are filled up apparently and graft shows faintly extending between 10th dorsal and 2d lumbar (Fig. 4).

November 12, 1915. Child will take a few steps, but as deform-

ity has increased since appearance of abscess bed treatment is ordered continued.

Myringotomy, left ear February 9, 1916, and right ear February 11, 1916. Gram negative intracellular diplococci in smear, February 11, 1916. The outlines show the degree of the deformity.

Case 9.
1. January 13, 1915.
2. May 16, 1915.
3. September 9, 1915.
4. November 3, 1915.
5. January 25, 1916.



Case 10. A. E., 17 years old. Admitted July 16, 1915.

Pain in right upper quadrant of abdomen and right lumbar region; was well and strong until 5 months ago, then coughed up blood; began to lose strength and weight. Examination, negative for pulmonary tuberculosis though repeatedly made.

Four months ago pain began in above areas; sneezing caused great pain; this increased to date of admission. Loss of weight then 16 lbs. No tuberculosis, cancer, rheumatic, cardiac, or pulmonary history in family. Marked kyphosis from 6th to 11th dorsal; extreme tenderness; slight lateral curvature to left in dorsolumbar region. Knee jerks not obtained.

Operation July 17, 1915. Incision from 5th to 12th dorsal spines curving to left in center. Spinous processes of 5th to 12th dorsal split with broad chisel well down to laminæ. Esmarch applied to leg. Graft taken from left tibia (7 x 1 x 1/3 in.), periosteum retained by motor saw. Fitted to spinal groove and held in place by kangaroo and chromic sutures, plain gut sutures for skin. Sterile dressing; spinal assistant brace over this; plaster cast over leg dressing. Gas-oxygen anesthesia. Healing

uneventful. Cast removed from leg September 5 ,1915. Patient allowed out of bed in brace.

October 9, 1915. Graft shown clearly uniting 5th dorsal and 12th dorsal inclusive (Fig. 5). Fusiform area of increased density on each side of vertebræ in this region. Periosteitis? Abscess? Disease involves 7th to 10th dorsal vertebræ inclusive.

October 20, 1915. No pain, tenderness, or edema. No psoas resistance; knee jerks normal; no anesthesia; walks quite well; general health excellent.

November 26, 1915. Patient returns from a convalescent home with radiating pain at site of kyphos and increase of deformity. Says he has had no accident. Coughing considerably. Wears brace not perfectly applied. *X*-ray taken identical with that 6 weeks ago. Graft in place and of good density.

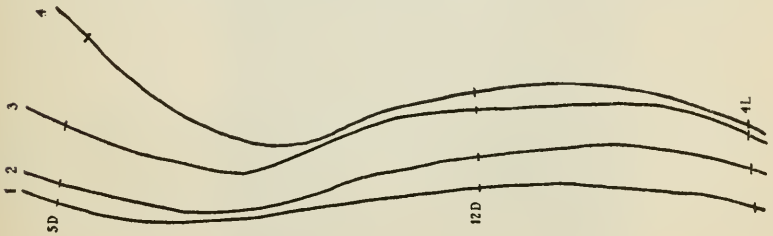
December 21, 1915. Since coming again under regular treatment pain has disappeared and patient says he feels better than before operation. Deformity, however, remains the same. No exaggeration of knee jerks; no psoas spasm. Chief complaint is feeling of weakness in back.

June 30, 1916. No pain; slight cough. Tuberculous orchitis with abscess for several months also tuberculous infection of right elbow. Wears brace; general condition, fair; knee jerks not exaggerated; deformity has increased coincidently with appearance of secondary lesions mentioned. Graft shown uniting 5th, 6th, and 7th, also 10th, 11th, and 12th; bone too dense between 7th and 10th to distinguish graft. Opaque mass at sides of 9th to 12th, possibly abscess.

Case 10.

Operation July 17, 1915. Graft 5th to 12th dorsal

1. September 11, 1915.
2. October 20, 1915.
3. December 21, 1915. Note swelling at sides of vertebræ
4. June 30, 1916.



Case 11. L. C., 28 years old.

January 10, 1910. No tuberculosis in family. No personal history of tuberculosis, pneumonia, pleurisy, typhoid fever, or other illness. Seven years ago fell down stairs, but was not thought to be injured. For the past two years, however, has carried herself badly, had considerable pain in lumbar region extending to right thigh. Has pain at night a good deal, and finds she cannot lift anything well and tires on walking soon. Has lost weight lately. Examination showed a well marked kyphosis at 10th, 11th, and 12th dorsal, with also a well marked lateral deviation to right at this point. No psoas spasm; no pelvic induration; no enlarged glands; no tumor in breast or elsewhere; knee jerks exaggerated. A plaster jacket was applied and she was able to do her work with very little trouble or pain and without increase in kyphosis for over five years. In May, 1915, however, without known cause there was an increase of pain and weakness with return of exaggerated knee jerks and deformity became more marked.

April 12, 1915. Under ether anesthesia, the spinous processes were divided with a Hayes saw and chisel from the 9th dorsal to the 2d lumbar. On account of the lateral curve the groove passed from the left of the center below to the right of it above. The kyphosis was also very sharp, the 11th and 12th vertebræ being fused into a mass smaller than one vertebra. It was very difficult to fit the graft over this sharp angle and in doing so, the graft split. However, it was made to fit well and held firmly in place by kangaroo sutures. The muscle and fascia were pulled well over graft with chromic gut, and plain gut was used for skin. Over the dressings, a spinal assistant brace was applied. Healing of back and leg uneventful.

June 18, 1915. *X*-ray (Fig. 6) shows graft from 9th dorsal to 3d lumbar, also fusion of 11th and 12th dorsal and 1st lumbar. There is an opaque mass at sides of vertebræ from 4th dorsal to 1st lumbar.

July, 1915. Was allowed up, but felt weak, and was nauseated for some days after, so put to bed for two months more. Since then has been up daily from one to, lately, four hours. No exaggeration of knee jerks. A projecting point of graft causing painful pressure, this was removed November 15. It was found to be

Fig. 5

Fig. 6

Fig. 7

firmly united with split spinous processes, and bled from cut surface, showing it was living bone. It was the upper end of lower fragment and did not seem to be united with upper fragment. The adjoining ends were, therefore, freshened, and again covered over with muscle and fascia, and brace reapplied over dressings. Patient is in excellent condition.

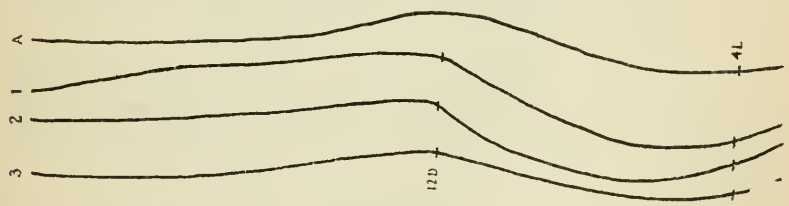
December 17, 1915. There is still some radiating pain on forced inspiration, but it is at level of 6th vertebræ well above diseased area.

February, 1916. Graft visible except at 12th dorsal where bone is too dense to distinguish it. Smaller opaque mass at sides of 6th to 12th vertebræ.

June, 1916. Discharged from hospital. Walking well. Occasional pain radiating about 9th dorsal; no cough; no exaggeration of knee jerks; general health excellent. Has a chronic synovitis of left knee since walking, about six months. Wears spinal brace still.

Case 11.
1. January 10, 1910.
2. April 9, 1915.
3. November, 1915.

(A) Lateral curve before operation. Note swelling at sides of vertebræ



Case 12. T. M., 35 years old.

For three and a half years, has had pain in spine. Paraplegic, February, 1913. Albee operation, November 12, 1913. Periosteum retained on graft. Casts put on and patient kept in bed till March, 1914. Paraplegia disappeared, allowed to begin walking. Cast replaced by spinal brace June, 1914.

November 25, 1914. Walking about well; no pain except occasional stitch radiating across lumbar region when getting up after lying down a long time. Has gained about 40 lbs. Got in fight with drunken man to-day and put his weight into him, throwing him to ground, and falling on him, etc. Two or three

days after fight began to have radiating pain across lower lumbar region about eight times a day, and felt spine more stiff. No loss of power in limbs.

Examination showed no induration. Reflexes at knee increased; no ankle clonus; no increase in deformity. Hyperextension of thighs 5°. Some deviation of trunk to left. No tenderness over spine. Flexibility not less than before apparently.

December 1, 1914. Pain has diminished 50 per cent. in the last week.

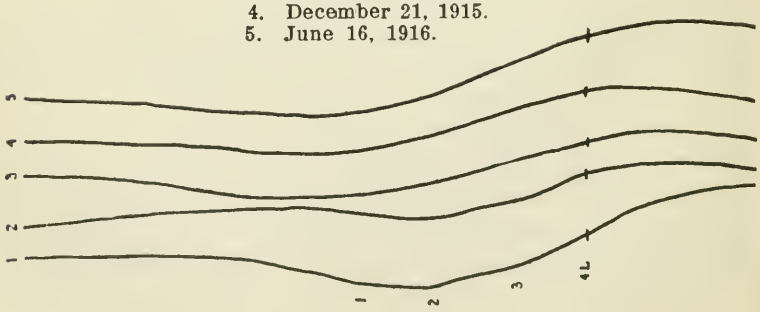
April 16, 1915. Pain occasionally across top of pelvis, knee jerks not exaggerated, walks stiffly, rather hyperextended, but works all day, wears spinal assistant.

December 21, 1915. Very rarely pain now. No exaggeration of knee jerks; no pelvic fulness; no psoas spasm. Wears spinal assistant. Works all day. Walks 4 to 5 miles a day. Weight 168 lbs. *X*-ray shows no graft, but bone is too dense to distinguish detail above third lumbar. There is a fusiform opaque mass on each side of the vertebræ from the 9th dorsal to 2d lumbar. This appears in a number of the plates by the site of operation, and may be callous or possibly abscess, with very thick walls, which gives no other evidence of its presence. The 5th lumbar appears atrophic, and at this point begins the lateral tip of spine which was noted as long as a year ago.

June 16, 1916. Weight 169 lbs. General health excellent. Wears brace yet. Has rarely, perhaps every two days, a momentary radiating pain about crests of ilia. Works daily; no psoas resistance.

Case 12

1. May 29, 1913.
2. November 12, 1913.
3. April 16, 1915.
4. December 21, 1915.
5. June 16, 1916.



Case 13. M. C., 20 years old.

Had pneumonia at 6 and 9 years of age. Four years ago thrown violently on back from bicycle, unconscious half an hour, then rode home five miles. A month later an appendix, which had ruptured, was removed. This was followed by pneumonia and empyema which was drained. A rib was excised two months later for better drainage, also an osteomyelitis of a metacarpal was operated upon at the same time, and again twice later. Four months later, a right tibial subperiosteal abscess was opened, and three months later a similar abscess on left humerus. Four months after the fall the kyphosis was noted by mother. Two years later a large left pelvic abscess was opened just above Poupart's ligament and has been discharging very freely ever since. Blood examination showed 3,400,000 red cells, hemoglobin 55 per cent., 19,000 white cells (66% polynuclears and 34% lymphocytes). The kyphosis has gradually increased in spite of braces, which were applied about this time. Increasing weakness in back, night cries, and loss of weight with a slight cough for the last year. Urine: 1,020, trace of albumin, no sugar, no casts. When I first saw her the 2d, 3d, 4th, and 5th lumbar vertebræ were involved; there was also some right lateral curvature of spine here. Knee jerks exaggerated. A second large abscess pointing in right groin. Patient was eager for the operation.

July 26, 1915. I took from right tibia a large strong graft, with its periosteum, by the motor saw, an Esmarch having been applied, and fitted it closely into the previously prepared groove in spine from 1st lumbar to 3d sacral segment. The hemorrhage was moderate. My assistant meanwhile closed the leg wound in the usual way, removing Esmarch after this, inserting several capillary drains and covering with rubber tissue; then the dressing and plaster cast, toes to knee. The next morning the foot was blue and cold and painful. The cast was cut down, foot elevated, warmth applied and cast strapped on lightly. The next day cast was removed, considerable hemorrhage had occurred in wound, the pressure probably interfering with the circulation in the leg. The retention sutures were removed and the wound partly opened, and warm applications kept up. During the next month there was sloughing of part of the soleus and anterior tibial muscles, also ulcers on the heel and little toe. These con-

tinued very sluggish until they were exposed constantly to electric light when they closed with remarkable rapidity.

The spinal wound closed by first intention, a subsequent pressure sore of skin also healed well. The graft is firmly in place, and the amount of discharge from pelvic abscess has diminished one half. The pain has left, and the patient says she has not felt so well in years and is gaining fast in weight.

November, 1915. Urine: 1.020, trace of albumin; no sugar; occasional granular cast.

Blood: 4,000,000 red cells; hemoglobin 50 per cent.

November 8, 1915. X-ray shows marked rotation of the vertebræ at site of disease, with the large dense graft in position. Tracings of kyphosis show no change since graft was inserted.

December 3, 1915. Developed phlebitis of internal saphenous on right side, with some temperature, swelling, pain and tenderness.

December 25, 1915. Very slight tenderness and swelling of the vein itself are the only signs of the phlebitis still present.

July, 1916. X-ray shows dense graft in place. No pain or increase of deformity. Still discharge from left pelvic abscess. Fifth and fourth metatarsal bones removed this spring for osteomyelitis of right foot. All other sinuses healed; general condition very fair.

Case 14. H. S., 15 years old. Admitted October 22, 1909.

Maternal grandfather and aunt were tuberculous. Child was perfectly well till five years ago; then had severe attack of diphtheria, and received antitoxin. No history of accident; no typhoid. Three years ago was three weeks in bed with pain in both hips thought to be rheumatism. Markedly affected by damp cold weather. Next winter had a similar attack with resulting lateral curvature of spine which was cured by exercises. When I first saw him, there was evidently osteitis of the lumbosacral spine with lateral curvature and compensatory increase of normal curves above diseased area. Pain in spine and sciatics, stiffness, loss of weight and strength. No other joints affected: no pulmonary symptoms. Skiagraphs reported to show nothing. Advised support, rest, life out of doors. Tuberculin test. Spent several months now in California, where he developed some pulmonary symptoms. Dr. Murphy of Chicago saw him while there, con-

firmed my diagnosis of Pott's disease and gave him same advice. April, 1910, when I saw him again, there were no pulmonary signs, but there was a slight systolic murmur at apex, the same spinal symptoms, and more loss of power and stability at lumbo-sacral region. Skiagraph showed rarefaction of 4th, and 5th lumbar and sacrum and no interspaces between these bones.

May, 1910. Jacket was applied and worn night and day, rest emphasized. Sleeping out of doors. General health always most carefully attended to. Left iliac abscess suspected September, 1910. Several falls about this time with but temporary increase of pain and stiffness.

March, 1911. Increasing in weight: no pain. Good support from jackets. Been rather quiet, but walks some without, he thinks, any bad symptoms resulting. Going to spend some months in California.

October, 1911. Skiagraph shows slight increase in lateral curvature, but no extension of disease. Has been free from pain except some in knees and hips in damp weather. No abscess found.

April, 1912. Lungs clear; heart normal; no change in kyphosis: no pelvic fulness. Not quiet enough, so from January 1 to April, 1913, in bed in corset at Miami.

May, 1913. A Van Winkle corset strongly re-enforced applied; gave excellent support.

September, 1913. No pain; no change in kyphosis; no abscess found. Corset supports well. To school. Felt well and was too active, and by December had pain in sacral region.

June, 1914. One interphalangeal joint became swollen without known cause, stiff and tender. *X*-ray show bones perfect.

September, 1914. Had severe attack of rheumatism affecting back and hips while staying in a damp place in Canada. Slight increase in kyphosis.

December, 1914. Marked increase in kyphosis and more stiffness and pain. Anemic. No abscess found. Urine shows a trace of albumin, and some hyaline casts. Blood showed 3,800,000 red cells: hemoglobin, 66 per cent.; 7,100 white cells: polynuclears, 65 per cent. Went South for warm climate, to be quiet out of doors. Wearing corset which fits well.

March, 1915. Pain in legs and back, stiffness of lumbar

muscles increased quickly without known cause a month ago and great loss of stability at lumbosacral junction. On examination now, abscesses are found in both gluteal regions with psoas resistance and increase of kyphosis. Pain and weakness. No exaggeration of knee jerks. Five years and a half of protection with casts and braces, attention to general health, very much more restricted activity and long periods of bed rest have not controlled disease. In consultation, one surgeon advised drainage of abscess, curetting of vertebræ, and prolonged rest in bed; another, one or two years rest in bed; another, Albee's operation and rest in bed.

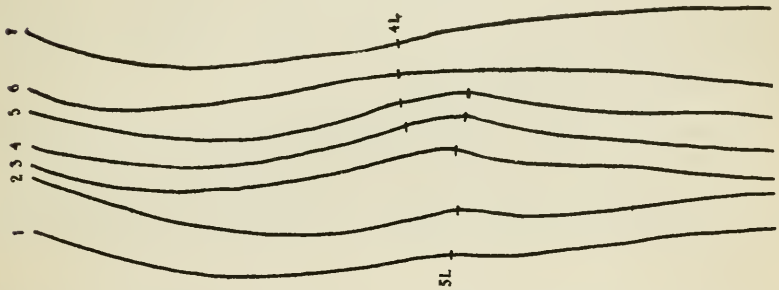
May 20, 1915. Gas-oxygen anesthesia. Dr. Albee assisting. Semicircular incision 1st lumbar to 4th sacral. Broad chisel to bisect spines and widen this groove. Over sacrum the groove was shallow but considerable force was used to broaden and roughen the subperiosteal surface. Considerable hemorrhage. With motor saw then removed graft (8 x ¾ x ½ in.) from tibia (Esmarch having been applied). This came out nicely with periosteum and fitted well after clipping top and bottom a little. Sewed in with heavy kangaroo gut and completely covered, fascia had to be incised in places to allow this to be done. Plain gut skin sutures. Over the dressing a plaster turtle back spica previously made was applied to immobilize. Time of operation, 80 minutes. Recovered at once in good condition. Plaster cast over leg.

Wounds healed promptly and cast was retained four months. There have been three serious attacks of nephritic pain in both kidneys lasting three, four, and seven days, accompanied by nausea, pain, gas, and depression. Urine at these times contained blood, pus, and casts, was but little diminished in amount, sp. gr. 1015-20. Eye grounds were negative. The large gluteal abscesses have been aspirated four times and there is considerable residue, but the pus is thinner. Cultures negative.

December 23, 1915. (Fig. 7) He is now in very good condition, without pain. Keeps very quiet in bed, only because told to do so, as he feels he could move about without discomfort. Outline taken today over graft which is firmly in place and not tender. Leg pain less and bone gap filled up. No temperature. A phenolsulphonephthalin test returned 100 per cent. in two hours.

June, 1916. No sign of abscess; graft firm; no pain; has been walking about some without support to back and feels very well. Has occasionally some traces of blood and albumin in urine. A month ago one of the small chips of bone inserted about end of graft was removed as it projected somewhat and caused a painful bursa. Healing prompt and permanent.

Case 14.
1. September, 1910.
2. July, 1911.
3. May, 1913.
4. September, 1914.
5. March, 1915.
6. November, 1915.
7. July 15, 1916.



Case 15. H. C., 56 years old. Admitted September 26, 1913. Duration of disease, 27 months. Twenty months before admission, the pain radiating from lumbar region to stomach was very severe. Nine months ago, the kyphosis was noted and has gradually increased. Legs became weak and for last two months has been unable to walk at all. Knee jerks exaggerated, ankle clonus, some disturbance of sensation in limbs, some difficulty to void urine. There was, however, an enlarged and painful right kidney to complicate symptoms and increase distress. Wassermann negative. Von Pirquet positive. Dr. Lyle asked me to see the case and I advised fixation of the diseased area with bone splint.

October 4, 1913. Dr. Lyle operated as follows: incision to left of spines 9th dorsal to 1st lumbar. Muscles and fascia raised and retracted from spinous processes and laminæ as far as articular processes. Periosteum also raised and retracted outward. Incision over crest of left tibia, and piece of bone 20 cm. long by 2 cm. wide by 1½ cm. thick removed with Gigli saw. This piece of bone was fractured in the middle to secure good fit and then put along

left side of spinous processes which had been prepared. The spines of the 10th, 11th, and 1st lumbar were drilled, and kangaroo tendon was passed through the drill holes. This was then carried over the bone splint and through the muscle and fascia external to it, then tied, so holding splint firmly in place. Volkmann splint applied to leg and felt jacket to spine and shoulders. *X*-ray showed 11th and 12th dorsal vertebræ involved.

December 30, 1913. A large cystic kidney was removed.

January 19, 1914. Knee jerks markedly exaggerated, complains of pain in sides at level of spinal lesion. A very difficult patient to control. To-day found lying in bed with head raised and spine unsupported. Fitted spinal brace with great difficulty. Was discharged from hospital March 25, 1914; improving fast. Walking in March, 1915, a few steps alone, and quite a distance with support. Wearing her brace still.

September 26, 1915. Has improved very much in past few months. Can go up and down stairs and walk four blocks unaided. The family think the deformity has not increased. General health good. There is still some pain in right side and back below kyphosis.

Case 16. O. Q., 28 years old; fireman. Admitted November 13, 1913.

Six weeks before admission, fell from roof and fractured 1st lumbar vertebræ. Immediately after accident, sensation was diminished a good deal in lower limbs. Two weeks after the accident, the kyphosis was first noted. On admission, I advised fixation of this area by bone graft.

Dr. Lyle operated as follows: Curved incision 12 cm. made over 12th dorsal and 1st and 2d lumbar vertebræ. Fascia and muscles freed from spines and laminæ to articular processes on left side and the periosteum raised to expose the bone. Second incision made on anterior surface of left leg for about 15 cm. and a piece of anterior surface of tibia sawn off about 8 cm. long by 1.5 cm. thick. This piece of bone was laid on the left side of spinous processes, and kept in place by kangaroo tendon sutures which were passed through holes drilled in spinous processes and then through the muscle and fascia on the outer side. Felt jacket put on back and moulded splint on leg. *X*-ray showed a

distinct lesion of the first lumbar vertebræ with partial destruction of the body.

January 26, 1914. *X*-ray showed graft in place, ¼ in. to left of 1st to 5th lumbar spines.

March 31, 1914. Discharged from hospital cured. Brace was applied when he was allowed up and taken off July 1st, 1914. Patient has been working on fireboat from April, 1914, to June, 1914, when he was assigned to duty at headquarters.

October, 1915. No psoas spasm; no pain or tenderness; can bend forward and touch fingers to ground with knee straight. Walks with quick light gait without limp. Outline 1 shows deformity.

December 23, 1915. *X*-ray shows no graft. Twelfth dorsal, 1st and 2d lumbar are solidly fused and 3d lumbar is partially fused to 2d. There is a slight lateral angulation at 1st lumbar and a slight kyphosis at 1st and 2d lumbar. Patient strong and well. Examination same as in October, 1915.

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(1) The Prognosis of Pressure Paralysis. Times and Register, November, 1890.

FOREIGN BODIES IN THE RESPIRATORY TRACT*

NATHAN W. GREEN, M.D., AND LEON T. LE WALD, M.D...

Whenever a condition has been shown to be amenable to successful treatment, although that condition has long been recognized as a distinct pathological entity, it receives renewed attention from the part of the profession most in contact with it particularly, and from the profession in general, later on. This has been shown to be the case with one abdominal condition after another —too numerous to mention—and has been observed in other regions, particularly the chest and its organs of approach. We are here concerning ourselves with foreign bodies in the larynx, the trachea, the bronchi and the terminal mechanism for the interchange of oxygen, viz., the lungs.

These objects all at one time or another have connection with the air current and act differently in the tissues from bodies which are buried completely. Any tendency to encapsulation means encapsulation with an opening at one or more points; and this capsule forms more the wall of a cavity in which the body lies free and in the midst of pus which is evacuated from time to time, than a true encapsulation.

History.—Going a little into the history of this class of cases it appears that the surgical attack of foreign bodies in the upper air passage dates back as far as Hippocrates who devised catheterism or intubation of the larynx in order to relieve the suffocation. Poulet[1] says the idea of opening directly into the passages was first advanced by a "Roman physician named Asclepiades, who had no opportunity of putting it into practice and was long exposed to the raillery of his contemporaries and successors."

Transverse section between two rings of the trachea was proposed by Antylus, Oribasis, Aetius, and Paul of Egina, because

* Reprinted from *Annals of Surgery*, June, 1916.

Fig. 1.

Röntgenogram of bronchial tree of female child, nineteen months of age. Bismuth mixture injected through bronchoscope. Consolidation of right lung prevented complete injection. (Lynah and LeWald.)

of the then current idea of the impossibility of the recovery of divided cartilages. Habicot prior to 1824 pointed out the way toward the rational employment of bronchotomy in cases of foreign bodies. According to Brünings and Howarth,[2] "the systematic examination and treatment of the trachea and bronchi by the peroral introduction of straight tubes was initiated by Killian in 1896. After being successfully applied in a case of a foreign body lodged in the bronchi it was recommended in 1897 as a clinical method." The names of Killian, Von Eicken, Brünings, Chevalier Jackson, Mosher, Ingalls, Yankauer and many others are all familiar in connection with this subject. Many of these investigators are laryngologists. But it is from the general surgical stand-point that we are interested in this subject this evening.

Anatomy.—In the laryngeal portion of the respiratory tract the vocal bands constrict the lumen. Between the false cords above and the vocal bands is the ventricle of the larynx. In this recess an object often finds lodgment. The next stopping place is at the level of the cricoid where the lower end of a pin or an elongated nut meat may become caught, as in two of our cases. Having passed the upper part of the respiratory tract the next point of arrest will be at the bifurcation of the trachea, if the body is too large to pass into one of the main bronchi. This site rarely arrests the object, however, as bodies small enough to pass the cords will slip into the bronchus. The right bronchus from its large size and the position of its openings is the one most frequently entered (Fig. 2). The spur dividing the two bronchi is situated to the left of the median line of the trachea and serves to direct a rapidly moving object into the right bronchus.

Less frequently it may pass into the left main bronchus. When the body has passed into either main bronchus its next point of arrest is either in the eparterial bronchus on the right or the hyparterial bronchus on the left or in the "trunk bronchus" on either side. The latter is fortunately the more frequent place. The trunk bronchus may be called the continuation of the right or left main bronchus after giving off the branches to the upper lobes.

A smaller body may penetrate to the divisions of the "trunk" bronchi," the various lobe bronchi, and their subdivisions.

Classification of Foreign Bodies.—The foreign bodies found in the respiratory tract may be divided according to their composition into mineral and metal and organic objects. Most of the mineral or metal objects on account of their density are impervious in a greater or less degree to the Röntgen rays. Of the organic objects those which have a certain percentage of calcium or other heavy salts are detected by the *x*-ray (pieces of bone and teeth). Again, foreign bodies may be divided according to their shape into round, elongated, irregular and sharp-cornered.

The most to be dreaded are the organic objects which are round and smooth and can swell rapidly,[3] such as seeds and beans, and those which break up easily, such as nut meats. Pieces of bone and pieces of tissue are also to be placed in the dangerous class. By far the most easily recovered ones are the metallic bodies.

The most frequent occurrence of foreign bodies in the air passages is in children. Brünings says 69 per cent. of foreign bodies occur in children up to twelve years of age. The greatest frequency is at about the age of two years. All of our personal cases have occurred in children, except one, that of the soldier who inhaled a badge fastener at the age of seventeen years and in whose lung it remained for six years (Fig. 3). So this particular disorder may be called a disease of childhood and youth.

F. S.,* twenty-four years of age. Patient was admitted to the medical service of St. Luke's Hospital under Dr. Austin W. Hollis, suffering from cough, with a foul expectoration. Seven years ago he had thoracotomy done in London for what appeared to be an empyema. The wound healed in five or six weeks and there has been no discharge since. The week previous to his admission he expectorated large amounts of foul-swelling pus, although coughing no more than usual. The sputum was greenish-yellow and there was never any blood in it. In short, he gave a clinical picture of a patient suffering from bronchiectasis.

On January 30 he was *x*-rayed by Dr. Truesdale, and this picture showed the presence of a foreign body. On January 31, and on three later days, he was *x*-rayed by Dr. Le Wald, who con-

* This case was presented before the New York Surgical Society, April 28, 1915, and was reported in the October, 1915, number of the Annals of Surgery.

Fig. 2.

J. P. Bead from necklace lodged in right bronchus. The bead was made of opaque blue glass.

firmed the diagnosis of a foreign body, and, by taking a picture at right angles, showed that the object was of the nature of a cotter pin. On going over the anamnesis, it was brought out that seven years before he had "swallowed" a badge fastener which he thinks he was holding in his mouth when a comrade slapped him on the back. (The badge fastener was used to fasten the regimental badge to his uniform.)

He was transferred to the surgical service March 20, 1915. Repeated attempts to find the foreign body were made by upper bronchoscopy, and, although the object could not be seen, in one of those attempts, the presence of a cavity was demonstrated, and in coöperation with Dr. Le Wald, the nearness of the end of the bronchoscope to the foreign body was shown. Later this helped to locate the pin by measurements and by the *x*-ray. On March 29 the bronchoscope was introduced and the magnet introduced through it, with the hope that the foreign body might be of iron; then the *x*-ray picture was taken. This showed the magnet to be within an inch of the foreign body, but there seemed to be no tendency to attract it. It was decided then that it would be better to go in through the back and remove it in this way.

On April 1 three shot were placed on the skin as a marker together with a piece of half-inch-mesh wire netting. A radiograph picture was then taken by Dr. Le Wald in two directions. The distance of the foreign body was measured and it was found to be 6 cm. from the shot in one direction and just in front of the rib.

Operation (April 1, 1915).—Under local anesthesia, two ribs were removed by Dr. Green. The lung was found to be adherent to the parietal pleura, as was expected, and to have a hard spot in it. At the suggestion of Dr. Martin this hard spot was entered with a needle and found to be the wall of a cavity. The needle was followed with a pair of dissecting scissors and upon opening them the cavity was widely opened. A pair of Blake's gall-stone forceps was then introduced into the cavity and the foreign body grasped and brought out. A tube was then placed into the lung and the wound was partially closed, leaving drainage.

The interesting points are that the *x*-ray located the badge fastener; repeated attempts to see the object through the bronchoscope failed, but the bronchoscopic method showed that there

was something causing granulations which filled up the right terminal bronchus. Second, that there was a cavity at the end of the right terminal bronchus, 2½ inches in length. (This was proved by passing a probe through this scope and feeling it "give" as it passed the opening of the cavity.) Therefore, the foreign body probably lay loose in this cavity. Third, that the foreign body lay well toward the base of the lung, behind, and just anterior to, the rib. Fourth, that the most of the cavity was rather behind the bronchus than just at the end of it.

The convalescence was more rapid than in the case of F. B. The character, odor, and quantity of the sputum changed markedly for the better, and the patient looked the picture of health.

It has been impossible further to report on this case, as the patient while crossing to England on the Lusitania was drowned at sea.

Classification of Cases.—The reaction of the organism to the various foreign bodies differs with the length of time of lodgment. Recently inhaled bodies produce at first but little reaction beside that of reflex coughing and dyspnea. This class of cases may be termed the immediate cases. Patients in whose lungs the foreign body has found lodgment for a longer time present the complications which make their condition that of extreme severity. They may be classed as deferred cases.

This differentiation in the cases is important throughout the whole tract, but nowhere as important as in the deeper structure of the lung, where the late manifestations may be those of infection, either acute and local, or more chronic, with the changes in the general system due to absorption.

The Means of Diagnosis.—The first point in making a diagnosis is the history. This is often of an indefinite nature, especially in cases of long standing, and the essential points may be forgotten until the later symptoms may lead one to suspect the presence of a foreign object, when one again goes over the history with the patient's family (as is illustrated in the case of F. B.).

F. B. Patient was admitted to the surgical service of St. Luke's Hospital, special to Dr. William A. Downes, on December 16, 1914, suffering from a cough and fever. He was then six years of age. One month prior to his admission he began to

Fig. 3.

Röntgenogram of chest of F. S., showing foreign body, badge fastener or cotter pin, and showing bronchoscope and through it magnet passing into cavity where object lay.

cough, but was not ill enough to go to bed. His cough continued and grew worse, and five days before admission he grew markedly worse. He had lost weight and complained of pain in the right chest. Upon admission, he gave the signs and symptoms of a right empyema, for which he was operated upon immediately. As his condition did not clear up in the usual time, he was *x*-rayed by Dr. Le Wald and the presence of an upholstery tack in the region of the right bronchus was discovered as the cause of the delayed convalescence. Upon questioning the father more closely, it was recalled that the child "swallowed" a tack two years previously, but this had been forgotten. He was turned over to Dr. Green for bronchoscopic examination and for removal of the foreign body, if possible.

The physical findings at that time were dulness over the right chest posteriorly from the angle of the scapula to the base, with diminished voice and breath sounds and numerous large, moist râles. An attempt was first made by means of an electromagnet, introduced into the persisting sinus in the right chest, to draw out the tack, but this failed. Repeated bronchoscopic examinations through the larynx showed granulations at the end of the right bronchus with pus exuding. The cavity which proved later to be surrounding the tack was not entered at this time. A tracheotomy was performed on March 2, and seven days later the boy was bronchoscoped through the tracheotomy wound. At this time the bronchoscope passed nearly to the cavity and forceps could be pushed into the cavity for two inches.

It was considered that part of the tack at least must be made of iron and so a small electromagnet, which passed through the smallest size of bronchoscopic tube, was made, and this was introduced through the tube ten days later, under combined ether and cocaine anesthesia. The tack was mobilized by this means and brought up into the end of the bronchoscopic tube with the head of the tack across the lumen of the tube. An attempt to grasp it failed and the procedure was then at once repeated. This time it brought the point of the tack into the bronchoscopic tube. It was then grasped by special forceps through the tube and, resistance to its removal being felt, a steady traction was made upon it as the child breathed, and little by little the resistance was overcome and the bronchoscope and the forceps through it grasp-

ing the task, were removed simultaneously. There was some bleeding from the granulations. A suction tube was at once introduced to remove this, and in a moment the bleeding stopped.

After the operation the child steadily improved. The character of the expectoration which at first was fetid and green changed to a white expectoration without odor. The amount of expectoration diminished as well as the amount of drainage through the chest, and the child gained flesh.

The interesting points in the case are that the foreign body remained in the lung for two years and that there was no attempt at encapsulation. It lay in a cavity in the lung at the end of the right terminal bronchus, and this cavity contained about an ounce of pus.

The later history of F. B. showed that his health greatly improved, while the side was open and draining, but that there was loss of flesh and color when it closed. The expectoration also again became foul and thick and reverted toward his previous condition, before removal of the foreign body.

He was readmitted to the hospital, August 3, 1915, and on August 20, 1915, a thoracoplasty with excision of the seventh and eighth ribs, under combined general and local anesthesia, was performed. The pathological findings at this time were as follows: There was a sinus tract leading from a scar in the skin through the seventh rib. The seventh rib had a large spur attaching it firmly to the sixth rib. The sinus tract led down to the dome of the diaphragm and also apparently connected with the bronchus. The pleura was but imperfectly adherent to the chest wall and the pleural cavity was opened at this time but was closed by suture.

On December 28 the external wound was again healed.

This condition of affairs is more apt to obtain when the object has found lodgment in the smaller bronchi and there has become fastened with no further displacement. An object which remains in such a position after the initial symptoms of severe cough and varying dyspnea may give rise to comparatively little disturbance until some of the deferred phenomena have begun to show themselves.

On the other hand, an object which remains stationary in the larynx or trachea will probably give rise to more or less constant

symptoms, especially those of dyspnea, either from occlusion of the air interchange or from a continuous reflex inhibition of respiratory movements. "If the interference with the entrance of air is not great the foreign body will be tolerated and, particularly if fixed, give but few symptoms."[4]

Again, if an object has been arrested at the tracheal bifurcation or in the right or left main bronchus, it may be of such proportions that it is neither impacted nor coughed out, but acts as a movable body, which may be blown about by the current of air and find impingement upon different parts of the mucous membrane, each time causing a fresh paroxysm of coughing.

Finally, any symptom for which the cause is obscure must, among other things, be considered as possibly due to a foreign body. The presence of a localized bronchitis, a pneumonia which fails properly to resolve, a lung abscess, a bronchiectasis, or a chronic empyema should lead our attention to the possibility of the complication of a foreign body, and one should at once proceed to a further means to clear up the diagnosis.

The most important next step should be to have the patient submitted to a röntgenologic examination. This is important in immediate cases and also in those of the deferred type. Chevalier Jackson has said that it is his belief that almost all kinds of foreign bodies can be detected by a properly taken röntgenogram.[5] This is in a large measure true; but it has been our experience that such a radiograph may not in itself give strong enough proof of the existence of an extraneous object (Fig. 4), but may be of assistance in suggesting the location of the foreign body and become a record when one has finally recovered the object. It is difficult to conceive how a seed or a nut meat in the bronchus can be of sufficient density to betray, in the immediate cases, its location.

After the Röntgen-ray and of equal importance, but secondary in sequence from a diagnostic stand-point, comes the direct inspection of the larynx, trachea and bronchi by means of the bronchoscope. One is fully aware that he may have laid himself open to criticism in placing this procedure second in point of time to the röntgenologic examination, but the reasons for doing so are the following:

First, the Röntgen-ray examination requires but little time.

Second, it may be done without an anesthetic (which in some cases is necessary).

Third, it is of no discomfort to the patient.

Fourth, it may materially shorten the following bronchoscopy by giving the operator a distinct indication of the portion of the bronchial tree or entire respiratory tract to be investigated.

All these indications may be altered in certain cases and the examiner who is familiar wth bronchoscopic work is the best judge in each particular case as to which shall precede the other.

Treatment.—In the matter of treatment of these cases, Garré and Quincke say: "When we consider the not infrequent unfortunate consequences of foreign bodies which have not been removed (hemoptyses) and the most unfavorable prognosis of operations on the secondary pulmonary suppurations caused by them, the necessity of an earlier active therapy needs no stronger argument."[6] With them it is one's privilege to agree as also with Von Eicken, who says: "Whilst in earlier statistics the mortality in cases of aspiration of foreign bodies showed a terribly high percentage, at the present time it is an exception for anyone to die from such a calamity if suitable treatment is available."[7]

The treatment of the immediate cases and that of the deferred cases differ markedly, both as to procedure and as to the ultimate outcome. An immediate case which is treated unsuccessfully at the first sitting, or if necessary sittings, becomes then a deferred case and passes at once into the category of serious illnesses.

It is not the purpose of this paper to go into the minute and tedious details of bronchoscopic technic, but rather to indicate what in our opinion are the salient points of the bronchoscopic procedure, which entails, to a large degree, the sum of immediate treatment.

In the immediate cases the object of the treatment is to get at the foreign body and remove it through the natural passages. (This paper is not dealing with foreign bodies forced into the respiratory tract by solution of continuity of the chest wall.) To do so one must first have a suitable bronchoscope and then be sufficiently familiar with its use to introduce it successfully. It can be done with local anesthesia or under general anesthesia or by the aid of a combination of both. Children in our experience require a general anesthetic for the introduction of the tube

Fig. 4.

H. G. Fish bone lodged in trachea. The shadow corresponds in size to the bone which was expelled through the tracheotomy tube.

through the larynx. A combination of general and local anesthesia with preponderance of the latter may be used if the tube is to be introduced through a tracheotomy wound. Adults submit to upper bronchoscopy under local anesthesia with very little discomfort.

In immediate cases if the first or second trial prove unsuccessful, especially in fragmented objects of an organic nature, a tracheotomy should at once be done with the introduction of the bronchoscope, and, failing to find the object, a large tracheotomy tube should be inserted in the hope that coughing may bring out the offending material. This expedient in the case of J. P. might have given a favorable outcome as it did in the case of H. G.

J. P., an infant, was admitted to the surgical service of St. Luke's Hospital, April 18, 1913, with the history of having aspirated a glass bead from a necklace. Before admission two unsuccessful attempts had been made by an outside physician to enter the bronchoscope through the larynx. The cause of the failure was due to the relatively small size of the rima glottidis when compared to the size of the tube used.

When admitted the patient was suffering from pneumonia, and on account of her extreme condition no attempt was made again to pass a bronchoscope. An *x*-ray was taken, but before more could be done the patient died. No autopsy was allowed.

H. G., aged two and one-half years, was admitted to the surgical service at St. Luke's Hospital, November 14, 1914, with the history of having "swallowed" a fish bone. An *x*-ray was taken which gave the suspicion of a shadow in the upper part of the trachea. Esophagoscopy and bronchoscopy failed to reveal a foreign body at this time. The patient was kept under observation for a few days and then, as he showed no further symptoms was sent home. He was brought back December 3, 1914, suffering with slight dyspnea, and an intubation was performed by the House Surgeon, Dr. Wells. After a short time another attempt at bronchoscopy was deemed advisable and, on account of the increasing dyspnea, tracheotomy was prepared for. On attempting to pass the bronchoscope the child ceased breathing. With the assistance of Dr. Steele tracheotomy was rapidly done, and upon opening the trachea the patient coughed out through the tube a slender piece of bone three-quarters of an inch long.

After that he made an uninterrupted recovery and was discharged on December 22, 1914, tracheotomy wound healed.

If the attempt to remove the foreign body has been successful in these fresh cases there need be but little fear of further complications, and the patient may be allowed to leave the hospital on the following day, if the peroral route alone has been used.

If the attempt has been unsuccessful and the object has not been coughed out after a tracheotomy, then the case passes on into the next class, that of deferred cases, unless the approach through a posterior bronchotomy be considered advisable and the patient's condition warrants it.

The development of symptoms in this transition stage is awaited with the gravest forebodings, for at this time begin the complications. These may be pneumonia, gangrene, lung abscess, and empyema, in the order named. Any one or all may result in fatality. Or there may be ulceration of the mucosa with perforation and a surrounding cellulitis or abscess formation and sepsis. Or there may be stricture of the bronchus and the development of a bronchiectasis. In these conditions should the patient survive the primary acute destruction of tissue, we have remaining a chronic condition with its connective tissue changes, in the center of which still remains the foreign body. Patients with these late complications then belong to the deferred class of cases and the foreign body present, which has acted as the exciting cause, still continues to act as an aggravation of the pathological condition until removal.

If the acute complications are survived the final condition of the untreated cases resolves itself into that of a chronic lung abscess containing the foreign body. These chronic lung abscesses resemble bronchiectases, they contain much pus beside the foreign body. Their walls may be irregular or smooth and they always are connected with the outside air by one, or even two, openings.

The patients as far as their symptoms go are suffering from bronchiectasis. They have a profuse intermittent perulent expectoration with greenish, foul-smelling pus. Their cough is worse on lying down. They suffer from afternoon temperature, have a hectic look, but rarely if ever does the sputum contain tubercle bacilli. They have clubbed fingers, especially if the duration of their illness has been over a year.

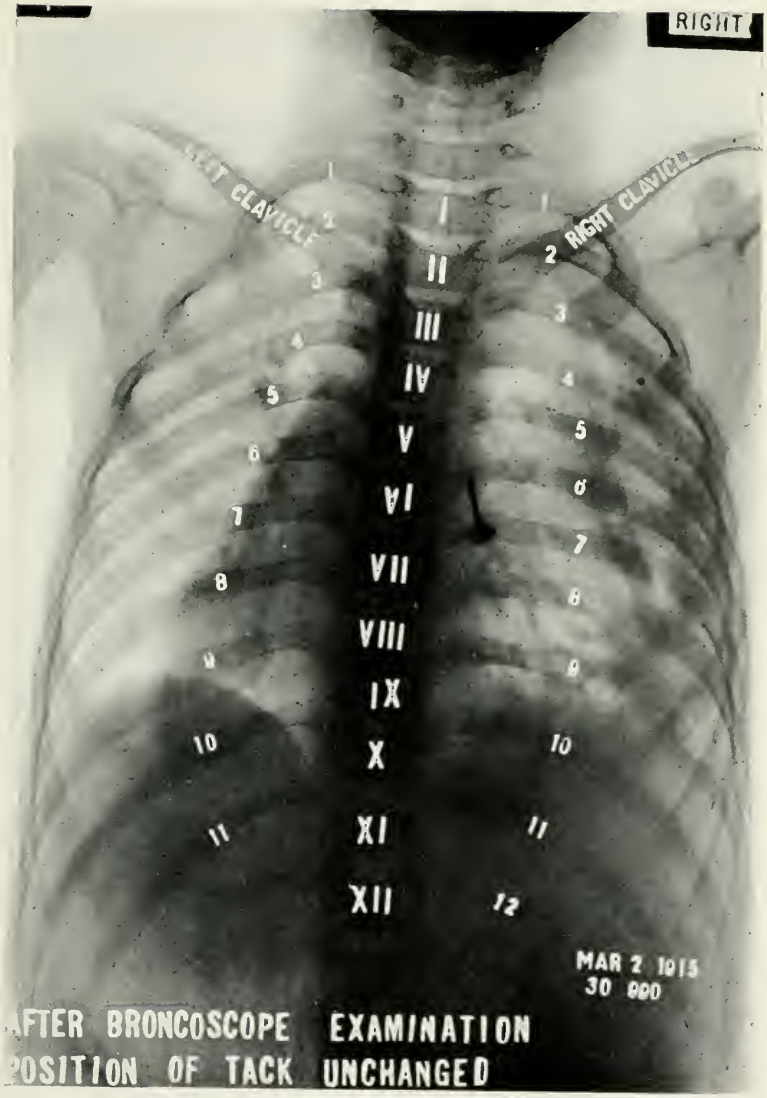

Fig. 5.

Röntgenogram of chest of F. B., showing foreign body, tack, in abscess cavity communicating with right bronchus, after an attempt had been made to reach it orally. It was later removed by a lower bronchoscopy.

The treatment of these deferred cases consists of removing the offending object, and of securing drainage of the purulent accumulation present. This will for a time improve the general condition, but the cavity caused by the foreign body will be very hard to heal. Many of these cases are opened from the outside and a bronchial fistula results. The purulent exudate is mostly discharged through the outside opening and, while doing so, the patient will remain improved. But when the outside opening is closed the patient again will return to a condition nearly as bad as before the removal of the foreign body. One case of ours, F. B. (Fig. 5), illustrates this well. Again there may be prompt healing of the outside wound with a great general improvement in the amount and character of the pus coughed up.

In general, the cavity formed by one of these foreign bodies must be treated along the lines of conservative surgery as applied to bronchiectasis. In a very comprehensive paper by Dr. Willy Meyer on "Bronchiectasis," both the non-operative and operative treatment of this condition are fully discussed, and one cannot do better than to refer to it for a complete history of the current procedure in this class of cases.[8]

Suffice it to say, it requires all the ingenuity one may possess in order to effect an obliteration of one of these lung abscess cavities. In the attempt to treat this condition surgically it is well to proceed by stages, and it is wise not to be too radical with each step. It may even be advisable to allow the patient to enjoy a reasonable degree of comfort rather than subject him to a severe procedure which may jeopardize his life, in the hope of a complete cure.

It is to be noted that in these chronic lung abscesses of long standing the amount of pleuritic adhesions present may be surprisingly and embarrassingly small. One must look to find them far less dense and less extensive than in the recent cases of pleuritic inflammation.

Summing up, the following conclusions may be drawn:

1. All recently aspirated foreign bodies should first be sought by the Röntgen-ray and the bronchoscope, without delay, and removed if possible through the mouth.

2. Failing to remove them through the mouth a tracheotomy should be done and another attempt made by means of the bronchoscope.

3. Failing in this the tracheal wound should be held widely open by wires or a large tube in the hope that the foreign body may be coughed out.

4. If immediate removal by these methods fail, a period generally elapses in which the patient may undergo secondary changes in the lung, such as pneumonia, gangrene, abscess, and generally an overlying empyema.

5. If the patients recover from these acute infectious processes, they pass into the class of deferred cases with the foreign body still present as an aggravating factor in their chronic lesion.

6. Removal of the foreign body in these deferred cases does not always effect a cure.

7. The lung abscesses must be treated along surgical lines and even then we cannot always hope for a cure, but rather only for an amelioration of their affliction.

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REPORT OF A CASE OF CONJUGAL SYPHILIS, WITH CONCLUSIONS*

By Edward Livingston Hunt, M. D.

Conjugal syphilis is of common occurrence. The literature of France and Belgium is especially prolific on the subject, as is evidenced by the reports of Fournier, Stillman and Rualin. The experience of most observers points to the fact that in conjugal syphilis the tendency is preeminently toward involvement of the nervous system. Many cases have been recorded of marital tabes and marital paresis, and marital cerebrospinal syphilis. This may, of course, arise from concealment, faulty diagnosis, or ineffectual treatment.

In passing, it may be of interest to note, that Fournier, in regard to the infection of women, always reached the same conclusion, namely that out of every hundred syphilitic women who had been infected sexually, eighty were immoral and twenty were married to men whom he found to be syphilitic, and again that twenty years ago cases of conjugal syphilis, in which one of the parties developed general paresis, were cited to call attention to the close connection that was then thought to exist between general paresis and syphilis.

The case which I am about to tell you of occurred in two old people, who had been married for thirty years. They had raised a large family, and had prospered. It was not until toward their sixtieth year that any symptoms of ill health developed. It was the wife who first displayed symptoms, although I am now satisfied, from a careful study of the situation, that the husband was the offender. These two old people, once they had become ill, began to lose very rapidly. This was especially true as regards their mental deterioration. The husband became confused, irri-

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table, unreasonable, and at times violent; the wife depressed, suspicious, and surly. He showed no physical signs, she very marked ones. The case, as it was presented to me, was as follows:

In 1910, Mrs. A., sixty-three years old. complained of stiffness in the legs, unsteadiness on her feet, difficulty in walking, and great mental depression. She had no interest in anything, refused to go anywhere, feared that she was ruined, disliked meeting people, and became irritable, unmanageable, and surly.

The physical examination showed unequal pupils, Romberg, a slightly ataxic gait, and absent knee-jerks. No cardia. There was no history of either alcohol or syphilis, and no suspicion of either. Her husband was perfectly well. The Wassermann test was negative. She was seen by several consultants here, who agreed that the diagnosis was probably a combined system disease, the cause of which was unknown. It did not seem advisable to give any mercury, at that time salvarsan was not generally used, and the symptoms which seemed to call most for treatment were the mental ones. Accordingly, the patient was placed in a sanatorium, with a good nurse, and given tonic treatment, irrigation, and baths. She improved very considerably in both physical and mental symptoms, so that it was generally conceded that the course of events had confirmed the diagnosis.

Three years later, in 1913, her husband, to whom she had been married for thirty years, had a slight stroke, followed by considerable and progressive mental impairment. From having been a very active and remarkable business man, he became childish, egotistic, self-centered, irritable, and somewhat demented. He could walk with difficulty, lost control of his sphincters and then displayed mental symptoms suggestive of Korsakoff's psychosis. He became disoriented, confused, and fabricated. There was no history of either alcohol or syphilis. The physical examination was absolutely negative. The Wassermann test on him proved negative. The urine showed from two to five degrees of sugar. The diagnosis appeared to be diabetes, plus arteriosclerosis. It was decided to try the luetin test. This gave at the end of seven days a positive reaction, that is, there appeared over the infected area two small red papules. The diagnosis was then made, one of syphilitic dementia. A lumbar puncture revealed a positive Wassermann, a positive globulin, and twenty-two cells. This patient was at once given hypodermics of the salicylate of mercury and intravenous doses of neosalvarsan, 0.4, 0.5 to 0.6 gram each. The improvement began almost immediately and continued slowly but steadily for over a year. He regained control of the sphincters, was able to walk, the sugar in the urine disappeared, and the mental condition returned to almost normal. Another Wassermann on the blood was made after the initial dose of neosalvarsan. This one was positive.

In June, 1913 Mrs. A., was then sent for, and another Wassermann was made on her. This proved to be indeterminate. A lumbar puncture was made; there were twenty-one cells. The reaction was positive to the Wassermann and to the butyric acid tests.

It was then decided to place her on antispecific treatment. She was given hypodermics of the salicylate of mercury every week for four months, beginning with one-half a grain, and increasing up to one grain. To this were added a good nurse, country life, and tonic treatment. She improved very steadily, the depression lifted, the peculiar form of mental aberration abated, the walking grew better, and she became stronger. At the end of four months, not sooner, she was given four doses of neosalvarsan intravenously. The first dose was 0.45 gram, the remaining three 0.6. These doses were administered two weeks apart.

She was then allowed to go home, and all treatment was suspended. At the end of two months, that is, in February, 1914, another lumbar puncture was made. This time the cell count was eight, the butyric acid test for globulin was negative, and the Wassermann test was indeterminate. The treatment of both mercury and neosalvarsan was then continued for another four months, with continued improvement.

As we can now look back on these cases with these complete records over a period of four years, it is possible to make a definite and comprehensive diagnosis. The husband was, of course, infected several years previous. He, in turn, infected his wife. The form which the poison assumed in him was that of a specific endarteritis, terminating in a syphilitic dementia. Added to this there was a syphilitic pancreatitis. The case of the wife was one of taboparesis.

These two people are now about 50 per cent. better than they were. The treatment has been pushed very steadily; it has consisted of hypodermics of the salicylate of mercury, from ¾ to 1½ grains, given weekly for three weeks, and then intravenous doses of 0.5 gram of neosalvarsan. Every three months the antispecific treatment has been stopped for a few weeks, and the patient has been given tonics. These cases are reported for various reasons. They teach the following facts:

(1) The importance of the *cerebrospinal fluid examination* as a routine measure in every neurological case. Had it been made at once in the case of the husband and wife, it would have made clear the diagnosis.

(2) The value of luetin. It was the luetin test which first made certain the diagnosis of syphilis.

(3) The importance and aid of a provocative dose of salvarsan. It was following the first dose of salvarsan that the blood Wassermann became positive in the case of the husband.

(4) The various forms in which the syphilitic poison may attack the system.

(5) The uncertainty of the Wassermann test when applied only to the blood.

(6) The value of combined treatment of mercury and salvarsan.

(7) The value of the intravenous form of administration of salvarsan.

(8) The very advanced age at which both of these patients were affected.

(9) The fact that reliance should not be placed upon the report of a single laboratory. No doubt in one of these two cases, if not in both, had two or three laboratories made the Wassermann test, a positive reaction would have been returned.

(10) The fact that all sugar in the urine is not of diabetic origin.

(11) The fact that the original source of infection became affected later than the second person.

(12) The absence of an aortitis in either case.

(13) The marked improvement which appeared in each of these cases, in spite of their advanced age.

THE USES OF THE HIGH-FREQUENCY CURRENT IN THE TREATMENT OF TUMORS OF THE BLADDER AND OTHER PATHOLOGICAL CONDITIONS OF THE URINARY TRACT*

By Henry G. Bugbee, M.D., F.A.C.S.

Although the high-frequency current had been in use for a number of years, it was not until its power to destroy tumors became an established fact that it became of practical value in its application to pathological lesions of the urinary tract.

The high-frequency current was discovered by two Americans, Tesla and Elihu Thomson (1). Morton (2) in 1881 produced the static induced current, non-oscillating in character, but the prototype of the high-frequency current. Tesla in 1891 produced the apparatus for very high-frequency effects which was improved a few years later by Elihu Thomson.

D'Arsonval's work in 1893 was solely with high potential currents and Oudin's with high potential, monopolar currents.

The modern high-frequency machine produces two currents: the d'Arsonval, which is a high-frequency oscillatory current with high voltage and relatively high amperage, a bipolar current; and the Oudin current, which is an oscillatory, high-frequency current with very high voltage but with much lower amperage, a monopolar current. An alternating current is supplied by direct connection, or by transforming a direct current. The alternating current enters a coil in passing through which the oscillations are increased. The current is stored in a series of Leyden jars, from which it is given off, the strength of the current being regulated by a rheostat and a spark gap.

These currents were early applied for therapeutic purposes. Large electrodes were employed which gave a diffused action.

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Some of the cases in which it was applied were: renal colic, with one electrode in the bladder and the other applied to the back, various types of cystitis, urethritis, urethral stricture, functional impotence, etc.

Bosquain (3) in his *Paris Thèses,* 1900, gave a history of the method of production of the high-frequency currents and their various general therapeutic uses.

Burch (4) showed by experiments the physiological action of the high-frequency current as applied therapeutically and gave indications for its use. These were varied and wide-spread.

Piffard (5), Snow (6), and Mabie (7) reviewed the history of high-frequency currents and their therapeutic adaptations, and Burch (8) added a few experiments.

Wright (9) in 1905 modified the construction of the high-frequency machine.

Somerville (10) successfully treated scrofulous ulcers of the eye with the high-frequency current, and Piffard (11) in 1906 made the first mention of the power of the Oudin current in destroying tissues, in referring to its use in the treatment of malignant or semimalignant tumors of the skin. He stated that the electrode applied to the skin caused little pain at first, but if retained in contact, the parts became hot and painful. The intensity of the current is under control and one can get an effect varying from a slight temporary congestion to absolute necrosis even in a few seconds in a localized area.

Geyser (12) compared the results obtained with the *x*-ray and high-frequency current in therapeutics; Piffard (13) described new high-frequency devices which were of interest from the standpoint of electrotherapeutics.

Riviere (14) wrote extensively of the treatment of superficial neoplasms by high-frequency sparks and effluves, and stated that he believed that superficial growths needed only this treatment. Deep neoplasms he also treated successfully when in centers accessible to electrodes, of which he had special ones for the stomach and bladder. He has used this treatment before and after operation and states that it is the method of choice in operable cases.

MacKee (15) reported twelve chronic cases of chancroids, as well as several acute cases cured by applications of the high-fre-

quency current. He also cured herpes progenitalis ulcers.

Cook (16) mentioned de Keating-Hart at the Paris Surgical Congress, December, 1907. He stated that the high-frequency current was more powerful than galvanism in electrolytic action, more rapid, affected indurated areas, and appeared to have peculiar selective action on morbid cells or cells of lower vitality as well as a stimulating action on healthy cells. He employed the current in reducing tonsils in adults, curing acne, moles, and papillomata. In removing scars he found it superior to the *x*-rays. Broken down sinuses healed rapidly under its application and hemorrhoids were easily destroyed.

Riviere (17) stated that in 1900 he called attention to the cytolytic action of high-frequency currents on neoplastic cells, and draws the following conclusions:

1. The high-frequency current cures small epitheliomata of the face.

2. It checks certain cases of malignant disease, especially lymphoid ones, by (1) thermo-electro-chemical action, and (2) by trophoneurotic curative action.

3. It is contraindicated in large tumors where excision is still the choice.

4. These operations on large tumors ought to be followed immediately by spark and effluve treatment, thus preventing contamination of the wound by carcinoma cells (leading to recurrences), as well as having a curative action on tissues.

5. It is the only means available in inoperable cases.

6. The great amount of nascent ozone liberated is disinfecting and nourishing to the tissues.

He obtained the best results in epithelioma, malignant chancre, rodent ulcer, and chancroid. He found it stopped the pain's progress—even with nodes involved—and cicatrized lesions. This holds for skin epitheliomata. In epitheliomata of mucosa it is palliative only, but relieves pain.

Riviere found that superficial sarcomata likewise yielded; the deep-seated ones resisted, but even here the progress of the growth was arrested, compression symptoms were alleviated and metastases ceased. It was more effective than *x*-ray in sarcomata, lymphosarcomata, and fibrous tumors.

In mammary cancer it helped to cicatrize ulcerating areas,

destroyed nodosites in skin and glands, and dried up foul discharges.

In incipient tuberculosis: lupus was cured by effluve alone, glandular tuberculosis was first attacked by monopolar or bipolar sparks and the treatment then continued by effluvation alone. Tuberculosis of bones and joints, fungous synovitis, periostitis, caries, with or without fistulæ, showed good results; swelling, pain, and muscular atrophy disappeared. He cited some cures of tuberculous orchitis.

Judd (18) treated nævi from the size of the little finger-nail to that of a half-dollar. The Tesla current was used, the patient holding one electrode; the other, a hollow glass rod with copper wire running through and projecting one-sixteenth inch beyond the end of the tube, was held far enough from the surface of the nævus to produce a heavy bombardment of sparks one-eighth to one-quarter inch in length. This was used for one and a half to three minutes, twice a week for three to twelve treatments. The nævus becomes a dry slough, separating at the end of two to six weeks, leaving a smooth reddened epithelial surface beneath.

He further cites its use in keloid, localized gangrene, where a rapid line of demarcation is desired, in perforating ulcers, reduction of enlarged tonsils, destruction of superficial epitheliomata and warty growths.

Riviere's article (19), "Cystolyse alto-frequente et fulguration du cancer," reviews the work on cancer but presents nothing new.

In 1909 mention is made that Marion (20) presented a case of tumor of the bladder treated by fulguration before the Paris Surgical Society. No details or results were given.

Riviere in 1909 (21) in his reply to de Keating-Hart made the following assertions:

1. The high-frequency current should be used at operation to aid the surgeon's knife.

2. The high-frequency current is the only therapy for inoperable cases.

3. The high-frequency spark and effluve exert specific elective action on neoplasm cells of cytolytic power.

4. The high-frequency spark and effluve stimulate the powers of resistance of healthy tissues.

He conclusively proves his priority in the application of the high-frequency spark for the destruction of tumors.

While treating a man for a disease of the nervous system, Riviere (22) asked him to move near one of the wires of the soleroid cage. On one of his fingers was a lesion which had not healed during several years; the wound healed in ten days. Then began his work of applying the high-frequency current, especially the Oudin current, in the treatment of various diseases. He found the use of the condensing electrode of Oudin to be analgesic. In certain skin epitheliomata the effluvial action was more manifest than that of the spark, and it was an aid in operating through the electrochemical action and by the nascent ozone produced.

Thus up to 1910 the high-frequency current had been discovered and it had been found that this current producing from one to two million oscillations per second when applied as a bipolar (d'Arsonval) or monopolar (Oudin) caused a certain beneficial therapeutic action on the tumors of the body, and if applied over a small area produced a destruction of tissue. It had been applied to destroy tumors, benign and malignant, and had been applied in the bladder.

However, it had not been used extensively in the urinary tract until Beer (23) in 1910 introduced the electrode of the Oudin current into the bladder through the cystoscope, and thus opened a way to destroying, under sight, tumors of the bladder. He reported two cases, one an inoperable tumor of the bladder in a woman of 81, and the second a papilloma in a woman of 66. The wire was passed through the cystoscope, the spark applied directly to the growth, at first blanching, then blackening the tissues with the production of gas, the growth rapidly disappearing after several applications of a few seconds each several days apart.

Following the case reports of Beer, Keyes (24), and Buerger and Wolbarst (25), each reported three similar cases with the same results. In one of the latter's cases a single application of a few seconds sufficed to destroy the growth. Buerger and Wolbarst called it a modification of the de Keating-Hart fulguration. They called attention to the ease with which it could be applied and its superiority over the thermocautery.

In 1911 the writer (26) reported a case of recurrent vesical

papilloma, the original growth, the size of a hen's egg, having been removed two years before. A recurrence the following year was destroyed by radium applied through a cystoscope, and the recurrence the following year was cauterized by four applications of a few seconds each of the high-frequency current. Here in one patient the various methods could be compared. The latter method has proved far superior from the standpoint of time of treatment, pain, recurrence, and end-result, there having been but one very small recurrence at the end of three years, and a second one a year later. Each small recurrence was easily destroyed by a single application of the Oudin spark.

Beer (27) in 1911 had collected 38 cases up to that date, all of which had progressed favorably under treatment with the high-frequency current.

The spark was applied by Buerger (28) to destroy cysts and hypertrophic conditions of the mucous membrane of the vesical neck and posterior urethra as well as small papillomata and fold formations in the urethra.

The heat effects of the high-frequency current range from hyperemia to burning (Clark, 29). Between them there is a desiccation point. This effect is produced by a high-frequency current concentrated to a fine metal point. The induction coil high-frequency current does not render possible an absolutely constant thermic degree, so he uses a static machine of large output. He treated small warts and moles successfully, then applied the current to epitheliomata, exuberant granulations, skin pigmentations, acne pustules, *x*-ray keratoses, lupus and bladder papilloma through the catheterizing cystoscope. He concludes that: (1) It is a valuable adjunct to surgery. (2) It helps prevent recurrences in cancer. (3) It sterilizes tumors. (4) It is useful in recurring cancer of the breast. (5) It is better than cautery in cancer of the cervix; is styptic, deodorant, and penetrating.

Further observations by Keyes (30) in 1911 on the cauterization of bladder tumors led him to make the following suggestions: (1) The smallest spark gap possible should be used, as a large gap causes unnecessary pain and hemorrhage, and also burns the insulation off the wire quickly. (2) The duration of a single treatment should vary according to the patience of the patient,

the danger of burning the bladder when only a small bit of tumor remains and according to the destruction of the insulation. If kept on too long he obtained a short circuit through the cystoscope. (3) Burning off of insulation may on withdrawal of the wire peel off a piece of rubber which drops into the bladder and becomes a nucleus for a stone. To avoid this he withdrew the wire and telescope together. There was no danger of this if the bladder emptied itself. (4) Intervals of one week were ideal for applications to growths. The slough separates in from two to four weeks. (5) Among accidents likely to happen, he mentions detachment of insulation, and in one case there was a severe hemorrhage and a swelling up of the bladder mucous membrane, simulating infiltrating carcinoma.

He found the Oudin monopolar superior to the d'Arsonval bipolar current. He found the results disappointing in cancer, prostatic hypertrophy, bladder ulceration, and enlarged verumontanum. In bladder papilloma 8 cases were cured and 2 were still under treatment.

Thomas (31) in 1912, reported 4 cases of bladder tumors treated by the high-frequency current by the method of desiccation. He condemns the removal of a piece of the tumor for diagnosis as it invites metastases and one cannot usually get enough for a sure diagnosis. He distinguishes between desiccation and fulguration, the former being a continuous effluve of current of low amperage from extremely high voltage, producing dehydration of tissue, resulting in blanching, devitalization, and drying. Fulguration is a superficial, blackening, charring, and cauterization of tissues.

Of the 4 cases reported, one had remained cured for one year, the pathological report of which was probable carcinoma. A second case had just been discharged, the result not known; a third case had been under treatment for five months, and a fourth case had been under treatment for two months, both of which had just been discharged.

The technique employed by Thomas was as follows: The spark gap was 1 to 3 mm. The best gauge is the effect on the tumor; no spark should be visible. A small bubble of hydrogen gas shows when the point of the electrode meets tissue; this is followed by a blanching of tissue, due to dehydration or oxidation result-

ing in complete devitalization. The dead tissue sloughs in a few days.

The current should not be used oftener than twice a week, usually once a week, and frequently every two to three weeks, according to cell reaction produced.

Clark (32) recommended that desiccation be used in superficial destruction; that it be applied with a single electrode in contact with the tissue and sparks of great length through air gap on to tissue, the other pole being grounded. For deep destruction he used the bipolar method, the metal point in contact with tissue, the other pole somewhere else on the body. This current devitalizes by drying the tissues; the spark is not hot enough to cauterize, but causes rapid dehydration of tissues, rupturing the cell capsule; it penetrates to one inch or more, according to the frequency, distance, time of exposure, and density of the tumor. It does not open vessels or lymph-channels; it sterilizes tissues. A dry crust separates in three days to a week. He employed this method in warts, moles, angiomata, various ulcers, acne, growths of the bladder through a catheterizing cystoscope, rectal papillomata, ulcerations, larynx tumors, and certain forms of eczema and parasitic skin diseases. Fulguration is a method (Clark) that should be used in combination with operative measures; even in advanced cases of bladder tumors treated by operation and fulguration, patients have been free from recurrences for from one to five years.

De Keating-Hart maintained that radiosensitiveness of tissue was in direct proportion to its temperature, the warmer the part, the more intense the action of the x-rays. For deep tissue beneath the skin surface, he used thermopenetration or diotherapy. To control dermatitis he used a leather or aluminum filter with cracked ice between two layers of gauze over the skin, or the surface was moistened and fanned.

Beer (33) in 1912 reviewed 183 cases treated for papillomata of the urinary bladder collected to that time, nearly all giving favorable reports.

In regard to the technique, Beer thought that copper wire was better than steel, as it produced more extensive necrosis, and that experience might show that other metal electrodes would be better than copper, also that other mediums would be preferred

to water and that the bipolar current might replace the monopolar, but doubted this as the d'Arsonval current had less cauterizing and less electrolytic action. He found that Nature seemed unable to divest herself of necrotic villi where a cystitis was present with a papilloma, and that the cystitis must be cured first. He treated no malignant growths except small superficial ones. He treated all other cases except where the tumor was inaccessible or the patient intolerant. In some cases where the whole bladder was studded, evidently only complete cystectomy would give relief.

Furniss (34) thinking he was dealing with a broad base papilloma fulgurated it. Tissue came away and a urethral calculus came through. He considered fulguration as the bloodless method of relieving impacted stone. Opposing Beer's statement that only benign growths were suitable for the high-frequency current, Rytina (35) reported the case of a carcinoma of the bladder kept under control and practically cured.

Judd (36) in 1912 reporting the results of treatment of tumors of the urinary bladder at the Mayo clinic, cited 17 cases treated by Oudin current of 114 reported. Eleven of these were cases of recurrence after operation. One case had gone fifteen months with no recurrence; 5 cases one year with no recurrence. No original non-papillomatous growths were thus treated, but recurrences were treated with considerable success. They found that villous growths on small pedicles were the most favorable for the high-frequency current.

In contradistinction to his early experiences in which he stated that the high-frequency current had no effect on carcinoma, Keyes (37) reported a case of recurrence after the removal of a growth, reported carcinoma which was treated and controlled by four applications of d'Arsonval current and four of Oudin during a period of eighteen months. The patient died of intercurrent disease.

Another case, supposedly carcinomatous by its appearance, disappeared entirely after treatment by the Oudin current and there was no appearance of growth for six weeks at the time the article was written.

Reviewing his experiences with the high-frequency current, Beer (38) in 1913 cited his former articles. He stated that he

found it better to have no air-gap between the electrode and lesion, that he got a whiteness at the spot of application, then carbonization; hydrogen was freely generated; metallic copper was present in the tissues. Beer used copper electrode, Wappler machine, street current, but this must be alternating or if direct must be transformed. Instead of an induction coil and interruption, the latest model used closed magnetic field transformers (step-up) giving more rapid oscillations and capable of being employed in any room.

In making an application he pushed the electrode in among villi for fifteen to thirty seconds at each place. The nearer the electrode approached the base of the growth the shorter the application was made lest the bladder wall be injured. If the bladder was touched it caused pain. Repeated applications were made to different spots until the whole growth was destroyed. The slough was voided in small pieces. This extended over several months in larger growths, but usually a few days to one week. The rheostat was used with half resistance on, sometimes all resistance on, the spark-gap one-eighth to one-fourth inch, usually a short gap.

He used an electrode of No. 6 Charrier insulated copper (at times steel) wire. As the rubber melted it was repeatedly trimmed. A total time of from three to five minutes' application was made at one sitting. In one case applications totalling ten minutes, thirty seconds, at twenty places were made, but this was a very large tumor. These were repeated in a few days. Treatments were discontinued as soon as the whole growth appeared necrotic. Sloughs were allowed to separate spontaneously or helped by bladder irrigations. After the base was thus exposed, after two or three weeks it was treated like the original outgrowth.

Beer cited the danger of perforating the bladder wall, but says this should not happen if one is careful. The reports collected by Beer of 33 surgeons were mostly favorable. There were 187 cases of intravesical papillomata and 20 cases of urethral papillomata treated in America and 28 cases in Europe. Definite cures for two years were controlled by repeated cystoscopy.

He stated that the high-frequency current had been used for fifteen years for the treating of superficial growths, and that its

only novel feature was its use under water in the bladder through the cystoscope. In selecting cases he gives as the contraindication: papillary carcinoma, patient's intolerance of the cystoscope, and growths inaccessible to direct and indirect cystoscopy, as well as those at the neck of the bladder which are traumatized by introducing the cystoscope, bleed and prevent accurate work.

Watson (39) in 1913, gave the following conclusions: (1) The treatment of benign tumors by high-frequency current is probably as effective and likely to be more effective than suprapubic excision. Many cases treated by operative methods both in papilloma and carcinoma recur after three years. The high-frequency current has only been in use a short time, so thus far one is only justified in feeling a strong hope that it is the best treatment. He considers the high-frequency current the best method in cases of papilloma. (2) This treatment should be abandoned as soon as it is evident that a recurrent tumor is malignant, and a transperitoneal resection or total cystectomy should be done. (3) It may be shown later that suprapubic excision is better for papillomata than high-frequency treatment.

Buerger (40) used the high-frequency current (Oudin) in the treatment of an ulcer of the bladder which was early cured, and in a second case in conjunction with mercury injections he obtained a like result.

A further report by Buerger (41) gave a recurrence in one case and he stated that he had tried the fulguration in two cases of callous ulcer without improvement and in a second callous ulcer he excised the ulcer.

As a means of facilitating the passage of descending ureteral calculi, Buerger (42) devised an olive-tipped electrode, the olivary tips being graded in size and screwed on. The dilatation was begun at No. 6 F., a current of 300 to 400 milliamperes being used for a few seconds. the second pole of the d'Arsonval current being placed at the back. Three stones respectively the size of a French pea, 8 mm., and 8 x 4 mm. were passed after this treatment.

Pilcher (43) found that with recurrent growths where new growths appear in their original form or spring from a new base after extensive removal of the tumor, the d'Arsonval current is better than the Oudin. A case was cured after operation and

after Oudin treatment had failed. One case of papilloma was cured. It puts carcinoma growths under control in inoperable cases.

Heitz-Boyer (44) gives as the treatment of large bladder tumors, a hypogastric incision, bladder tumor excision, and pedicle treated later by high-frequency current through the cystoscope.

Having employed the high-frequency spark for the treatment of vesical papillomata soon after Beer in 1910, and with the same good results, the writer (45) in 1911 began to employ it in cases of vesical obstruction where operation was contraindicated. It was found that prostatic obstruction, malignant, adenomatous, and fibrous, could be destroyed sufficiently to give partial or complete relief from the obstruction, such relief being permanent in some cases up to the time of reporting the cases in 1913. The technique employed was as follows: A No. 18 F. indirect close vision cystoscope was used. The current (Oudin) was applied with a No. 5. F. insulated steel wire passed through the cystoscope and held tightly against the portion of the prostate to be destroyed by means of the deflector. A one-fourth inch spark was used, the wire being held in contact with the prostate until the hydrogen bubbles ceased to form. A cut was burned through the obstructing tissue. At the same sitting or at subsequent ones, the cut was widened and deepened until the vesical orifice was freed posteriorly. Of 13 cases reported, 4 were carcinoma and 9 benign obstructions. The cases of carcinoma were advanced and inoperable. These patients lived one year or more, during which time they were able to void or pass a soft rubber catheter with ease when previously this had been impossible; they all died of metastasis or other intercurrent disease. The benign cases were median bar or small median lobe enlargements and two were cases of general adenoma. Those presenting obstruction from small amounts of prostatic tissue obtained complete relief of symptoms after from three to six applications. In cases of general prostatic enlargement the amount of residual was decreased. Reaction following the applications was slight; there was no bleeding. The operations performed under the eye, without shock, bleeding, or leaving a raw surface, seemed superior to

other methods in cases where it was necessary to destroy only a small amount of tissue.

Barney (46) reported a case illustrating the efficacy of the high-frequency current in treatment of tumors of the bladder, and called attention to a reaction in the mucous membranes around the base which he thought to be cancer. He excised this area, the pathological report showing only chronic inflammation.

Stevens (47) reported 2 cases of prostatic obstruction relieved by applications of the high-frequency current. The first, a probable constriction of the neck of the bladder, was relieved by four cauterizations of three minutes each in a three month period, the residual being reduced from 26 to 34 oz. to 9 to 13 oz. One more treatment reduced the residual to 1 to 1.5 oz., capacity 22 oz.

The second was a case of pedunculated median lobe. Six treatments destroyed this lobe. The residual was reduced from 14 to 1.5 oz. He thought that the d'Arsonval current could accomplish results quicker, but care should be used to avoid too deep destruction of tissue.

Ashcraft (48) in reporting a series of cases of benign and malignant tumors of the bladder treated with the d'Arsonval current through the operating cystoscope stated that he had found this current better than the Oudin. In experimenting he found that an application of 90 seconds of a 425 milliampere current with the rheostat at the third button burned a 1 cm. area 1 cm. deep in beefsteak. He found that it required a much stronger current under water than in air to penetrate tissues, and that it required a 425 milliampere current at least to destroy vesical tumors. He found that the Oudin current burned a very small area and only acted superficially, so he used the d'Arsonval. He considered the Oudin fit only for very small growths. He especially advises the d'Arsonval in large growths and where malignancy is suspected. His technique is as follows: The wire penetrates the tissues 1 to 2 mm.

First application: 15 seconds, 250 ma., then rest 15 seconds.
Second application: 15 seconds, 300 ma., then rest 15 seconds.
Third application: 15 to 40 seconds, 425 to 575 ma., with new wire.

As a rule there was a little reaction consisting of slight temperature, pain, frequency, burning, and distress. In carcinoma there may be more reaction. The amount of reaction is the guide

to repetition of treatment. He gives one week to ten days as the interval.

Four cases have remained cured eighteen months, sixteen months, four months, and three months, respectively; one case improved, died of uremia one year after; and one case was still under treatment.

In an exhaustive article appearing in November 1913, Young (49) reported 117 cases of vesical tumors of which 21, 17 per cent., were benign and 96, 83 per cent., were malignant. These cases were treated as follows: (1) suprapubic excision, 43, (2) fulguration, 19, (3) suprapubic drainage, 22, (4) suprapubic partial excision and destruction of base by cauterization or high-frequency current, 5, (5) no treatment, 28.

He stated that benign tumors were relatively infrequent, and unless cured, almost always became malignant.

1. (*a*) Suprapubic excisions .....47 cases
   35 malignant by microscope } 80 per cent.
   2 malignant clinically }
   12 benign (8 microscopically, 4 clinically), 4 recurred as malignant, 1 showed beginning malignancy, and 15 per cent. continued benign.
   (*b*) Excision with pedicle and portion of mucosa.....23 cases
   9 benign
   4 became malignant
   1 died of embolism
   2 cured four years
   1 result unknown
   1 extensive benign recurrence.
   14 malignant
   2 cures; 1 six years, 1 two years.
   12 recurred, with death in short time.

Young says, "These results are extremely bad; not nearly so good as obtained by fulguration, and show in a striking way the inadequacy of the suprapubic excision, even when great care is taken to avoid implantation and to thoroughly remove the tumor after clamping the pedicle."

(*c*) Excision of tumor with more extensive removal of adjacent vesical mucosa .....4 cases
1 case well after 2½ years,
2 cases had prompt recurrence,
1 died of carcinoma of liver and stomach 2½ years after operation.
(*d*) More or less extensive resection of entire thickness of bladder wall adjacent to tumor .....20 cases
3 benign
2 cured, one year
1 result unknown
17 carcinoma, 7 hopeless at operation
5 cured, one to ten years

1 operation, recurrence at one year
1 operation, recurrence at two and a half years
1 six years post-operative, nine years post-operative, inoperable, infiltration behind bladder.
1 cure, 5 years
1 cure, 1 year
1 cure, 1 year, died of uremia
1 cure, 1 year, died of recurrence 20 months
1 cure, 2½ years, recurrence 5½ years.

These results Young considers very gratifying.

2. Fulguration (d'Arsonval or Oudin)
12 benign cases treated through cystoscope
1 cure 6 months
1 cure 4 months
1 cure 6 months
1 cure 9 months
1 cure 15 months
1 cure 6 months
1 cure 18 months
1 cure 1 year
1 cure 5 months
1 cure 1 year
1 cure 4 months
1 case still under treatment.

Young believes these results show the great superiority of this method over suprapubic excision in benign cases, "especially as some of the cases were so extensive that the whole bladder practically would have to be excised."

The high-frequency current gave unsatisfactory results in almost all cases which proved malignant.

One case in which the high-frequency current was unsatisfactory is now cured one year after suprapubic resection of part of the bladder.

In four cases with partial destruction of the tumor by the high-frequency current there was an improvement in the frequency and difficulty of urination, but all died of metastasis.

In one case of carcinoma there was a wonderful disappearance of the growth.

In three cases splendid results were obtained by a combination of suprapubic partial excision, cauterization with Paquelin cautery and high-frequency current.

"It is possible," Young says, "to destroy malignant vesical tumors if the spark is strong enough and the bladder filled with air; so recently I have applied fulguration through an open-air endoscopic cystoscope. It is evident that extremely thorough cauterization, by Paquelin or electricity, can successfully destroy

vesical carcinoma if care is taken about preventing implantation and to thoroughly destroy the base of the growth."

Summary of carcinoma cases:

12 treated by cauterization through suprapubic wound.
3 are well.
1 small recurrence.
1 bladder free, retrovesical metastasis.
5 died.
2 rapidly losing ground.

Young's conclusions are as follows:

1. Visual excision is utterly inadequate and is followed in both benign and malignant cases by prompt recurrence.

2. Cautery is an extremely valuable agent with suprapubic or intraperitoneal operations. There are some brilliant cures, even in apparently hopeless cases.

3. Carcinoma, except extensive cases, is best treated by suprapubic resection.

4. For benign tumors, the high-frequency current seems thoroughly satisfactory but should be vigorously applied.

In tabulating further observations on the use of the high-frequency spark for the relief of prostatic obstruction in selected cases, the writer (50) added 8 cases to the 14 previously reported, making a total of 22 cases treated to August, 1913. Of the 22 cases, 8 were malignant, the disease being advanced, inoperable, and the obstruction complete. The obstruction in each of these cases was relieved so that the patients were able to void or partially empty the bladder and at times pass a soft catheter.

The benign cases, 14 in number, were as follows:

1. Small fibrous prostate constricting the vesical orifice, one case. The patient had been operated upon by perineal incision and stretching of the vesical neck with very little relief and partial incontinence since the operation. The patient was greatly relieved by a partial destruction of the prostatic collar with the high-frequency spark, ease of voiding, loss of frequency, and better control.

2. Median prostatic enlargement of prostatic isthmus, 2 cases, the symptoms were relieved and both patients emptied the bladder.

3. Small median lobes without general prostatic enlargement, 3 cases. Relief of symptoms, no residual.

4. Moderate general adenoma, one a case of diabetes where

prostatectomy was not to be considered, and a second where operation was refused. Symptoms disappeared. Residual eliminated.

5. Prostatic nodules left after incomplete prostatectomy, 4 cases. In all of these patients cystitis and atony were present. Three were improved, the residual diminished, and in the fourth the residual eliminated.

The experience of the writer led to the conclusions: that when a vesical obstruction was caused by a small amount of tissue, this could be destroyed and the obstruction relieved by application of the high-frequency spark, and that this was the method of choice in these cases. In no case was it undertaken when a general adenoma was present unless the patient's condition eliminated the possibility of prostatectomy or the patient refused an open operation. In the cases presented, (1) the cases of small fibrous prostate, median bar and small lobe obstructions, without general enlargements were symptomatically cured; (2) the other cases of obstruction, i.e., general adenoma and cases of incomplete prostatectomy with atony, were improved.

In the Year Book of the Pilcher Hospital, P. M. Pilcher (51) makes the following statements: "We believe that one can obtain better and more permanent results in treating bladder tumors by avoiding the use of the knife whenever possible. If the treatment is ineffectual through the cystoscope, a suprapubic cystotomy is performed, but no attempt is made to remove the tumor by resecting the bladder. Our present method of treatment consists in destroying the tumor more by actual cautery and deep penetration of the base with the bipolar spark." He considered the Oudin current best for ordinary papilloma and the d'Arsonval current best for recurrent growths.

Without mentioning the type of prostatic enlargement or obstruction, Beer (52) stated that the transurethral cauterization with Bottini incision as well as with the high-frequency current seemed to have only temporary effect.

Pedersen (53) reported a case of extensive adenocarcinoma of the bladder and intestines in which he used the high-frequency current without success. Also two cases of papilloma, one cured in four months and a second in two months.

Newman (54) says, "Operations employed for removal of neoplasms in the bladder are:

"1. Excision of tumor through suprapubic opening with knife or cautery.

"2. Partial resection of bladder wall.

"3. Total excision of bladder."

It is not necessary to refer to the method of removing growths per urethra with the aid of the cystoscope employed by Nitze and others, as the suprapubic route having many advantages is now always adopted. He reports two cases of simple papilloma operated by the suprapubic route, and one case of adenopapilloma treated by suprapubic excision.

Stevens (55) cited the two cases previously reported of prostatic obstruction relieved by high-frequency current and gave a third case of median bar where the residual was reduced from 3 to 6 oz. to 1.5 oz. Two other cases did not tolerate instrumentation, so the treatment was discontinued.

The residual was cut down from 5 oz. to 6 dr. in a case of transverse cicatrix after a suprapubic prostatectomy, by four applications of the high-frequency current by Bangs (56).

Uhle (57) considered the d'Arsonval current more penetrating than the Oudin. In two cases he noticed a recurrence after six months in treating a vesical papilloma with the high-frequency current. The recurrences were treated and they were free at the time of writing, one and a half months.

One case had been cured one year, another six months, two cases two weeks, one case was under treatment, one diagnosis doubtful; in one case of cancer the bleeding was controlled under treatment, and in three other cases of cancer the bleeding was controlled but the patients died later.

Clark (58) described desiccation as between hyperemia and carbonization, a rapid dehydration of tissues, rupturing the cell capsule and transforming it into a dry mass. He stated that recent experience justified the hope that in selected cases desiccation may be of service in prostatic hypertrophy treated by the urethral route. He also used the same method for urethral papilloma, caruncle, granulations, etc. The sterilization is somewhat deeper than the area destroyed on account of heat penetration. The advantages over cautery and chemical escharotics

are: (1) absence of much inflammatory reaction; (2) no contracted cicatrix, therefore less likelihood of stricture.

In the destruction of local tuberculous bladder ulcerations secondary to kidney tuberculosis which do not clear up after nephrectomy, Heitz-Boyer (59) employed the high-frequency current. He stated that it was necessary to destroy surrounding tissues for at least 1 cm. outside the lesion.

Bremerman (60) reported 31 cases of benign papillomata treated by the high-frequency current with one recurrence and this cleared up with the same treatment. He began at once to treat the base of the tumor and treated the whole base at one sitting.

Moloney (61) applied the high-frequency current to a calculus in a diverticulum of the bladder. After 56 treatments the calculus finally got into the bladder. He does not know whether the passage of the stone was due to the action of the current or to the dilatation of the orifice of the diverticulum.

It is now five years since the high-frequency current was first employed to destroy vesical papillomata, and following that for the destruction of other tissues in the urinary tract. It has been definitely proved that this current will destroy tissue superficially by actual cell disintegration when applied as a monopolar (Oudin) current, and more deeply when applied as a bipolar (d'Arsonval) current. The question is when and how to apply it.

In the treatment of papilloma of the bladder and urethra, the reports of 33 surgeons collected by Beer in 1914, showed that it was the method of choice in these cases. Reports since then substantiate this fact. Young's comparison with the treatment by excision is convincing. The writer has to date used the high-frequency current in 56 cases of vesical papilloma which were clinically benign. There have been recurrences in 6 cases. These recurrences were easily destroyed by further applications of the current. In one case of extensive involvement of the bladder wall (almost complete) the bladder was opened, the entire surface cauterized with the Paquelin cautery, and recurrences, which appeared almost at once, were treated with the high-frequency current. This patient died of anemia from hemorrhage. Following applications of the high-frequency spark to the pedicle

of a papilloma in another case, the bladder filled with blood-clots necessitating a suprapubic cystotomy, removal of clots, excision of growth, and cauterization of the base. There has been no recurrence in two years in this case. Application of the spark usually causes a cessation of bleeding.

The d'Arsonval current should first be applied to a papilloma, one pole buried in the villi, the other over the buttock. A current of 200 milliamperes is usually sufficient and should be given at repeated intervals, each application being sufficient to char the entire surface of the growth. When two-thirds of the growth has been destroyed, the Oudin current should be substituted and the destruction proceeded with more cautiously until the entire growth has disappeared. In working close to the bladder wall, an edema of the mucous membranes takes place which resembles infiltrating carcinoma. This disappears within a few weeks.

Any catheterizing cystoscope can be used for this purpose, and there should be no trouble with short circuiting. Aside from the two cases above mentioned, the remaining 54 of the writer's cases have progressed favorably, the entire growth being destroyed.

It is advisable in all cases of papilloma to make a cystoscopic examination once a year. Thus small recurrences may be discovered early and treated at once.

How shall we differentiate a benign from a malignant papilloma? This is often very difficult from the clinical and microscopical standpoint. Malignant papillomata are more often multiple, appear on the lateral and anterior bladder walls, have broader pedicles, appear more stocky, bleed easily, and cause more marked symptoms of vesical irritability and pain. The removal of a section for diagnosis is not always satisfactory. These tumors are often both malignant and benign and the section removed may not reveal the carcinoma.

Malignant papillomata do not react favorably to the high-frequency current. In 2 cases of the writer's in which it was employed, one showed signs of toxemia and metastases developed rapidly, the vesical symptoms were aggravated. In a second case, after two applications of the d'Arsonval current, the growth was covered with a slough, but the vesical symptoms were severe —frequency, burning urination, and pain in the bladder— and a wide resection of the bladder was made. In this case a wide

infiltration of the bladder had taken place and the question arises as to whether the application of the current had not hastened its spread. A third case treated by excision and cauterization has been free from recurrence for one year.

In diffuse carcinoma of the bladder wall, with painful, frequent urination, hematuria, and difficult urination, much can be done to relieve the symptoms by occasional applications of the d'Arsonval current. A current of 200 milliamperes is sufficient. The writer has applied this current using an olive, metal-tipped electrode in the bladder, the second electrode over the buttocks for a total of three minutes, at periods of a week apart until the bleeding is controlled, then a month or more apart, according to the symptoms. Other cases so treated by the writer lived for periods of from six months to two years, dying of metastases, during which time bleeding was absent, pain slight, and frequency lessened. All were able to void. The writer has 3 similar cases under his care at the present time; one for twelve months, one for eight months, and one for six months. They are all voiding without pain, have no hematuria, and are more comfortable than if they had submitted to an operation which would have amounted to a nearly total cystectomy.

The application of the d'Arsonval current by means of graduated olive-tipped bougies as suggested by Buerger, for dilatation of the ureter in assisting the passage of ureteral calculi, or the Oudin spark for the release of calculi lodged at the ureteral mouth, as applied by Furniss, is worthy of trial.

In a series of 46 cases of impacted ureteral calculi seen by the writer in the past twenty-eight months, in 6 the calculus was too large to pass, although located within the lower 5 cm. of the ureter, and was removed by open operation. No attempt was made in these cases to dilate the ureter. In another case repeated attempts have been made over a period of nine months to dilate the lower ureter; the calculus has moved to a point 1 cm. from the bladder, but has not moved from this position in four months. Two more cases were dilated, but have not returned for further observation and the result is not known. In 39 cases the calculi were passed. Of these 39 cases, 6 were treated by the passage and manipulation of filiforms, ureteral catheters, and the injection of oil into the ureter; in the

remaining 33, either the Oudin current was applied to the ureteral mouth with the wire electrode, or the ureter was dilated with olivary bougies with which the d'Arsonval current was applied. The d'Arsonval current probably aids in the dilatation and the Oudin stretched mucous membrane, thus enlarging the orifice.

In one case a cicatrization took place at the ureteral orifice, following repeated applications of the d'Arsonval current of 250 milliamperes. The cicatrix was stretched by a further dilatation of the ureter with the olivary tips.

The longer ureteral calculi remain impacted in the lower ureter, the smaller are the chances of releasing them. In assisting in their passage it is usually a question of changing the axis of the calculus, relying on the pressure from behind to force it on. This can be accomplished with a filiform or ureteral catheter. A dilatation of the ureter from below may be more easily accomplished with the olive tips. A calculus impacted at the ureteral orifice may be freed by destroying the mucous membrane around it with the high-frequency spark. The resultant damage is slight.

The application of the high-frequency spark for the relief of obstructions of the vesical neck in selected cases has, in the hands of the writer, proved a valuable adjunct. The cases must be carefully selected. As a method of choice it is best applied in those cases where the obstruction is caused by a small amount of tissue, as in hypertrophy of the mucous membrane, in chronic inflammation of the trigone and vesical neck in both the female and male, in median bar obstructions, cicatrices, small median lobe prostatic obstructions, and in small fibrous prostates. In these cases, the Oudin spark is used, the applications being made at intervals of a week or more, preferably at intervals of several weeks, until the obstruction is relieved.

In cases of general adenoma of the prostate, where operation is contraindicated by the condition of the patient, much relief can be obtained by burning through the prostatic obstruction and repeating the applications at intervals of six months or a year.

In carcinoma of the prostate, inoperable, with retention, much can be done to relieve the patient by destroying the surface of the growth, thus enlarging the vesical orifice. Frequency, tenesmus, pain, and bleeding are lessened and all but the frequency may disappear.

In 1913 the writer reported 22 cases of vesical obstruction treated by this means. Since then he has had 35 cases, making a total of 57 cases. They represent the following types:

Carcinoma of prostate and bladder wall	12
Small medium lobe obstruction without lateral lobe enlargement	7
Median bar obstruction	9
Small fibrous prostate	4
General adenoma of the prostate	9
Cicatrix at the vesical neck	2
Chronic inflammation of the vesical neck with hypertrophy of mucous membrane	4
Prostatic nodules remaining after incomplete prostatectomy	4
Tabes	3
Lateral lobe enlargement	3
	57

The cases of carcinoma were all inoperable. Some relief from symptoms was obtained in every instance. Three are still under observation, the others have died of metastases or intercurrent disease. One case of complete retention from a hard diffuse carcinoma of the prostate and bladder wall, treated for the last time eight months ago, has been able to return to work, and has gained weight; the residual has been reduced to 3 oz. and there are no urinary symptoms other than urinating every three hours.

In 5 of the small median lobe obstructions, the symptoms have been markedly relieved—one is improved and the other case reports that he is about the same as before treatment.

In all the median bar obstructions as well as those due to cicatrix and chronic inflammation of the vesical neck, the residual has been eliminated.

The cases of small fibrous prostates have improved, two are still under treatment.

In the 2 cases of tabes, the residual was reduced in one case from 8 oz. to 2 oz., and in the other from 2 to 3 oz., to 1 to 2 drams.

In the cases of incomplete prostatectomies with nodules of prostate remaining about the vesical neck, partial relief was obtained in each instance, although all had atonic bladders with chronic cystitis.

The cases of general adenoma were all suitable for prostatectomies as far as the prostate was concerned. Five patients refused operation and in 4 the general condition of the patients prohibited it. Three of these patients have died of intercurrent disease. Two are symptomatically relieved. Three are still under

treatment and improved. In 8 the residual was reduced, the frequency lessened. In one case there was no improvement. This was in an exceedingly large prostate where manipulation even with a specially made cystoscope was exceedingly difficult. In this one case only, the d'Arsonval current was used. The patient has complained of pains in the joints since the treatment and it is probable that these symptoms are due to absorption from prostatic tissue destroyed and not cast off.

The cases of lateral lobe enlargement have shown little improvement, probably due to the inability to destroy enough prostatic tissue.

There are many factors entering into this method of treatment which should be observed.

1. The selection of cases.

a. Vesical obstructions caused by a small amount of tissue are most suitable.

b. Where one seeks a partial relief of symptoms in an inoperable case or where operation is refused.

2. Technique.

a. Gentle manipulation.

b. The use of a small, close vision cystoscope with a deflector.

c. The destruction of a small amount of tissue at each treatment.

d. Treatments at wide intervals, only repeated when all symptoms from the former treatment have disappeared.

e. Treatment of cystitis if present.

The advantages of this treatment are:

1. An anesthetic is unnecessary.
2. The operation is performed by sight.
3. There is little or no pain.
4. There is but slight reaction.
5. There is no hemorrhage, and bleeding is stopped if present before applying.
6. No ulcerated surface or cicatrix is left after the dead tissue has come away.
7. The patient is not incapacitated.
8. The progress can be watched by the cystoscope.

The disadvantages are:

1. The length of time required to treat a case—often several months.

2. The necessity of destroying only a small amount of tissue at one time.

3. Increasing (temporarily) of vesical irritability where cystitis is present.

4. Difficulty of manipulation in some cases.

5. Amount of after-care necessary in many cases.

Papilloma of the urethra is rare, but when present can be easily destroyed by a mild Oudin spark applied through the close vision cystoscope or urethoscope. The writer has applied the spark with success in two cases.

In certain cases of hypertrophy of the verumontanum the high-frequency current may be applied with beneficial results. In one case of the writer's, one application of the d'Arsonval current (150 milliamperes) was followed by occlusion of the right ejaculatory duct and an acute seminal vesiculitis. The occlusion was relieved and symptoms disappeared in five days. In two other cases, the Oudin spark was applied through the close vision cystoscope with success.

For the destruction of herpetic ulcers, venereal warts, and the treatment of chancroids the high-frequency spark has long been used. A short Oudin spark suffices to accomplish the results and probably does this better than any other method of treatment.

SUMMARY

1. The high-frequency current is an alternating current of from one to two million oscillations per second. When used as a monopolar Oudin current it causes superficial destruction of tissue, or when used as a bipolar d'Arsonval current it causes deeper destruction.

2. Used in the urinary tract it is the method of choice in dealing with benign papillomata of the bladder.

3. Malignant papillomata and circumscribed carcinoma of the bladder wall are best treated by wide resection of the bladder wall and destruction of recurrences by the d'Arsonval current.

4. In cases of extensive carcinoma of the bladder wall, the growth may be retarded and symptoms lessened by the d'Arsonval current.

5. Certain types of vesical obstruction—due to a small amount

of tissue—may be cured by the destruction of this tissue with the Oudin spark.

6. Inoperable cases of vesical obstruction can be partially relieved by destruction of tissue about the vesical neck.

7. Failure will follow attempts to relieve such obstructions unless care is exercised in manipulations, and the treatments are given at wide intervals, a small amount of tissue being destroyed at each treatment.

8. The d'Arsonval current probably assists in dilating the ureter with the olivary bougie, and the Oudin spark may be an aid in releasing a calculus lodged in the lower ureter or at the ureteral orifice.

9. The Oudin spark may be of assistance in reducing a hypertrophy of the verumontanum or in destroying a urethral papilloma.

10. The Oudin spark is probably the best known agent for curing venereal warts, herpetic ulcers, and chancroids.

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REPORT OF A CASE OF CHRONIC MIDDLE-EAR SUPPURATION; THE RADICAL OPERATION; SINUS THROMBOSIS; EXCISION OF THE INTERNAL JUGULAR VEIN; RECOVERY.

EDWARD BRADFORD DENCH, M. D.

The patient was a male, twenty-four years of age, on whom a radical operation was done for the relief of a chronic suppuration of the left ear of many years' standing. At the operation considerable fluid pus was found in the mastoid antrum, walled off on all sides, and under pressure; this pus was in contact with the lateral sinus, thus constituting a perisinus abscess. During the course of the operation the sinus was accidentally wounded, the slight hemorrhage which followed being controlled by gauze packing. The ordinary meatal flap was cut; the upper portion of the wound was sutured, the lower portion being left open; and the entire wound was packed. Blood cultures taken immediately after the operation, and on the third and fifth days, respectively, were found to be negative. The patient's temperature remained normal until noon of the tenth day after the operation, at which time it was 101 F.: he complained of absolutely no discomfort and seemed to be perfectly well. On the evening of that day the temperature reached 104 F. and a blood culture taken at that time showed, when examined eighteen hours later, numerous colonies of the Streptococcus hemolyticus. The temperature fell spontaneously, after the first rise, to nearly normal; but on the afternoon of the eleventh day it again reached a point between 104 F. and 105 F.

The patient was immediately placed upon the operating table and the internal jugular vein was excised from a point just below the omohyoid muscle to the base of the skull. There was a clot in the vein above the origin of the facial vein, and also a clot in

the lateral sinus; the latter clot was thoroughly removed, so that free hemorrhage occurred from the torcular end of the sinus. The wound in the neck was sutured, with drainage at the lower angle, and the patient was returned to bed. The temperature fell immediately, and did not rise again above 102 F., this occurring, as I remember, on the day after operation. The recovery of the patient was uninterrupted; the wound in the neck healed by primary union; the radical cavity dermatized perfectly; and the patient is now in good health.

It may be stated that, beginning about a week after the operation, the patient was given several doses of an autogenous vaccine prepared in the laboratory; it seems quite possible that the rapid recovery, in this case, may, in part, have been due to the beneficial effect of this vaccine.

The case is of interest, chiefly, as demonstrating the importance of frequent blood cultures in cases where the sinus has been injured, or where it is suspected that a sinus thrombosis may occur, owing to the conditions found at the time of operation. We have, in the history of this case, a very definite picture of a sinus completely blocked by a clot as the result of traumatism at the time of the first operation; while this clot completely occluded the sinus no organisms could enter the blood stream. At about the eighth or ninth day, however, the clot began to break down: the blood current became loaded with pathogenic organisms; and usual temperature changes were evident, as the result of the toxemia. Prompt operative interference at this time completely removed the septic focus, and brought about an uninterrupted recovery. I might say that a blood culture, taken the day after the excision of the jugular, was negative, showing, in the first place, how promptly operative interference was instituted after the first evidences of blood infection, and, secondly, how possible it is by a complete and early operation, absolutely to rid the blood current of pathogenic organisms.

AN ACUTE OTITIS MEDIA.
EPIDURAL AND PERISINUS ABSCESS, FOUR MONTHS AFTER

W. G. BOWERS, M. D.

The following case is interesting as illustrating the insidiousness of onset in many acute inflammatory conditions of the middle ear, the difficulties of diagnosis, and the danger of such conditions.

F. W. W., aged thirty-seven years, was admitted to the Out-Patient Department complaining of pain in his left eye and left temple for the past two years. Four months previously, he had had a discharge from the left ear which lasted for four weeks. Since that time he had had some headache, but no pain in the ear, and his hearing had been normal so far as he knew. During the interval he had had four myringotomies, after each of which the ear had discharged for about a day, and throughout the entire four months he had had aching, tender spots in various parts of the left side of the head. He had been well all his life, excepting for soft chancre, twenty years ago.

Upon examination after admission to the clinic, the left drum membrane appeared to be thickened, with slight redness around the margin; otherwise the meatus was normal. There was no tenderness over the mastoid, but some tenderness was present well back behind the sinus. Hearing was normal to the whispered voice; there was slightly increased bone conduction on the left; Weber's test was negative; there was no spontaneous mystagmus. Caloric reactions were normal. Temperature was 99 F.

The patient was kept under observation for three weeks, during which time the tender areas and pain shifted from one place to another on the left side of the head; the temperature remained normal. The Wassermann reaction was negative. Eye grounds were negative. The first *x*-ray picture taken was negative, but the second, taken one week later, showed two dark spots, about the size of a dime, back of the sinus over the cerebellum.

When the patient was admitted to the ward the temperature was normal; the blood count showed 6,500 white blood cells; polymorphonuclears, 58 per cent.; lymphocytes, 54 per cent.; eosinophiles, 3 per cent. A smear made from the middle ear, after a myringotomy, showed Gram-positive cocci in pairs and chains.

On opening the mastoid what was apparently normal bone was found until the antrum was reached, where there were several soft cells and a drop of pus. It was decided to expose the sinus, and in so doing a cavity was opened from which some seropus gushed out. This cavity, which measured about one-half inch in width and one inch in length, was filled with granulations. In cleaning it up a sinus was found which led downward and backward, passing beneath a very large emissary vein, and opening into another cavity about the same size as the first one. The first cavity was over the sinus, exposing it for a distance of one inch and bathing it with necrotic material under pressure. The second cavity exposed the dura in the posterior fossa for a distance of one by one-half inches. The second cavity contained fluid of a more purulent character.

A culture from the antrum showed Gram-positive cocci in pairs and chains; but the cultures from the cavities were sterile. After thoroughly cleaning up the bone in and around these cavities, the wound was packed and partially closed.

Recovery was uneventful. The patient had no headache from the time he took the anesthetic. The wound healed in one month, at the end of which his hearing was normal.

This case illustrates a not uncommon condition during and following an acute otitis media. During the past year I have seen a number of similar conditions which had been watched constantly by very good general practitioners, but in which the diagnosis was not made until some complication had set in; in some cases not then. This was due, I think, to lack of appreciation of the fact that mastoiditis without temperature and without tenderness is a not uncommon condition, and that frequently it is these quiescent mastoids that show the greatest destruction and consequently the greatest likelihood of complications which, if they occur, are most often fatal. In one case which was being watched by two physicians, I made the diagnosis of mastoiditis

and advised operation within twenty-four hours. Because there was no temperature, very little tenderness, no swelling, no pain, excepting "neuralgia" in the top of the head, and a normal blood count, they disagreed. Eleven days later I was asked to see the patient again as a swelling in the external meatus and in the neck had been noticed; needless to say, the abscess, fortunately for the patient, had ruptured through the digastric fossa. There was very extensive necrosis with an exposed sinus and several ounces of pus extending into the neck.

THE REMOVAL OF DISEASED TONSILS BY A METHOD MINIMIZING HEMORRHAGE*

By Antonie P. Voislawsky, M.D., F.A.C.S.

Together With

A SKETCH OF THE HISTORY OF TONSILLOTOMY

By D. Bryson Delavan, M.D., F.A.C.S.

THE HISTORY OF TONSILLOTOMY

While the records of neither Assyria, Babylonia, nor Egypt appear to contain reference to this subject, the recognition of diseased conditions of the tonsils and the surgical treatment of the gland when enlarged must have existed from a very early period. Coming more and more into popularity during recent generations, few operations have been so frequently performed, with a greater measure of benefit to the patient, and, until recently, with less advancement in the positive scientific knowledge of the surgeon.

Theories innumerable as to the physiology and pathology of the tonsil have been advanced, and countless methods proposed for its treatment when diseased. Much controversy has therefore arisen regarding it, which, far from having been settled in our time by well-organized scientific investigation, still continues to hinder progress and to vex the professional mind.

Without question the urgent present needs in this department are, a clearer knowledge of the physiological value of the tonsil, recognition of the necessity for accurate diagnosis of its diseased conditions based upon recognized pathological principles, and, especially practical, scientific testing of promising methods of treatment, the careful comparison of the relative values of these methods as observed in large numbers of operated cases, and,

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finally, the long-continued study upon the individual of end results.

Of the various methods employed in recent years, radical extirpation of the tonsil by so-called enucleation has been heralded as the latest improvement, and has gained many advocates. Strange to say, the first mention extant made in recorded history of any surgical method for the removal of the tonsils refers to this very expedient; for Celsus, writing in the year 10 A. D., nearly twenty centuries ago, says: "Tonsils which remain indurated after inflammation, if covered by a thin membrane, should be loosened by working the finger around them and then torn out. But when this is not practicable, they should be seized with a hook and excised with a scalpel."

Aetius, no less than 500 years later, the next whose writings have brought us mention of the subject, assumes a position of far greater caution than did Celsus. He advises that the portion of the tonsil which projects, namely about one-half of the entire gland, may be removed; and he emphasizes the fact that "those who extirpate the entire tonsil remove at the same time structures which are perfectly healthy, and in this way also give rise to serious hemorrhage."

While Aetius could not have recognized the full import of his objection to the complete removal of healthy tonsillar tissue, he seems to have been the first to sound the warning against it which at the present time is claiming so much serious and intelligent attention; and thus at the very outset to have inaugurated the discussion upon "Tonsillotomy versus Tonsillectomy," a controversy which having flourished for 1500 years is still maintained with ever-increasing vigor.

In the ages following the time of Aetius, the interest of the profession in the subject waxed and waned, sometimes seeming to have altogether expired, and again being revived with more or less energy. But always the knowledge was vague, and the differences of opinion numerous and unreconciled.

Dionis, writing in 1672, opposed the removal of the tonsils because of his belief in their physiological importance.

As late as 1809, William Cooper, of London, in his Dictionary of Practical Surgery, describes the various methods of operation then in vogue, and says that the most popular were the knife,

the scissors and the ligature. His article ends with the statement that "the best modern practitioners for this operation prefer the common knife to any other instrument." During the twenty years following this statement of Cooper, interest in the subject became active in the United States. Several attempts were made here to improve the conditions of operation, and in particular to devise some satisfactory means by which the application of the silk ligature or the wire loop to the base of the tonsil to be ablated might be facilitated.

Much attention seems to have been awakened to the subject, especially in Philadelphia, Physick and Smith of that city, and Alexander Stevens, of New York, devising instruments of considerable ingenuity, generally of the ecraseur type. None of these, however, long survived.

In 1828, Physick gave to the world the invention which was destined to revolutionize the practice of the time, an instrument which has probably been more widely accepted, more extensively modified and more persistently utilized than any other contribution to practical surgery of the century. Perfected by the genius of Sir Morell Mackenzie, for many years it ranked without a successful rival; and to-day, as the so-called "Sluder modification," again heads the list in popular favor.

In 1832, Dr. W. B. Fahnestock of Lancaster, Pa., published a description of a ring-knife tonsillotome in which, contrary to that of Physick, the action of the cutting edge of the instrument was from behind forwards. This device was soon taken up by various instrument makers, especially in Continental Europe, the best known and most effective of its countless modifications being that of Matthieu, of Paris.

It is an interesting fact that the models of Physick and Fahnestock, made in this country at nearly the same time, have dominated the entire world of laryngology for nearly a century. Meanwhile, following them other workers were not idle. Time after time the discussion upon the removal of the tonsil was revised, perhaps nowhere more vigorously than in New York City under the influence of Dr. Horace Green, who in 1845-65 founded the science of laryngology and incidentally taught his generation the value of tonsillotomy, operating after the manner of Louis of

Paris with a bistoury or with scissors aided by a sharp-toothed holding forceps.

The prevalence of hypertrophied conditions of the tonsils of late years has been calling attention to this subject more than ever; while the method of operating under general anesthesia, inaugurated by Sir Morell Mackenzie about thirty years ago, and the still later discovery of local anesthesia, have opened the way for practically all of our modern improvements, and have enabled work of a thorough and satisfactory character to be performed. Strange to say, this seems to be the only feature of importance in this connection suggested outside of the United States.

As from the beginning, however, opinions relating to the general subject of the tonsil vary, discussion is active, and there is still much to be learned.

By far the most important question now at issue is that of diagnosis, for a clear distinction must be made between glands which are enlarged merely from temporary conditions of congestion, and those which are chronically hypertrophied and radically diseased. If, as many with good reason believe, the physiological function of the tonsil is a matter of serious importance in the general economy of nature, and, therefore, the organ should not be unnecessarily destroyed, especially at a time of life when its presence may be most needed as a factor in the normal development of the child, too great care cannot be taken to differentiate between cases demanding removal and those in which a proper course of treatment would effect a cure. Many of our clinicians would do well to acquaint themselves with the warning of Dr. John Noland Mackenzie, of the Johns Hopkins Medical School, against this crying evil, and to reconsider the dictum that every tonsil, from whatever cause enlarged, should be at once completely extirpated. Fortunately efforts are being made by Dr. Thomas R. French, of Brooklyn, and in various parts of this country, to improve the general knowledge of the subject and to evolve a perfect system of diagnosis and treatment.

This we also are trying to do at St. Luke's Hospital, where all patients are carefully examined as to the necessity for the removal of the tonsils before operation is advised, and where, through the liberality of the management, patients operated upon at the clinic are provided with beds until such time as they may be safely

dismissed. Under the able supervision of the Associate Attending Surgeon a method of operation has been developed which appears worthy of attention. A description of it by him follows.

THE REMOVAL OF DISEASED TONSILS.

When in January, 1912, the Clinic of the Department of Laryngology and Rhinology of the Out Patient Department at St. Luke's Hospital was formally opened the most important problem presented was the disposition of the many cases of diseased tonsils and adenoids which presented themselves. The Director of the Clinic had long believed and taught that the proper care of such patients after operation was an important feature of their treatment and that the custom of dismissing them at once from the Clinic and allowing them to return to their homes was unscientific and dangerous. He considered this especially true of those operated upon under general anesthesia. The Superintendent of the hospital recognizing the wisdom of this persuaded the Board of Directors to open a ward of seven beds in the Out Patient Department for the accommodation of children under twelve years of age. With this nucleus the operative work was begun. In November, 1914, the service was still further benefited by the opening of a male ward and a female ward in the main building of the hospital to adults and to children over twelve years of age.

Efforts were at once made to improve the technique of the operative work of the clinic. Tonsillectomy having taken the place of the old time tonsillotomy in cases requiring the thorough removal of the gland, our operations were begun by blunt dissection and then enucleation with the Mosley snare. As time went on other snares were used, but gradually the operative procedure followed the methods of Sluder and Beck in operations under general anesthesia, and that of Justus Matthews in local tonsillectomies.

Without criticism of other methods, in our hands the Beck instrument gives most satisfaction in the majority of cases, under anesthesia. The technique of the procedure which we have developed may be described as follows:

The patient is anesthetised with gas-ether anesthesia until the secondary stage is reached, and draped for operation in the usual manner. Lying prone on the table, without a pillow, the Den-

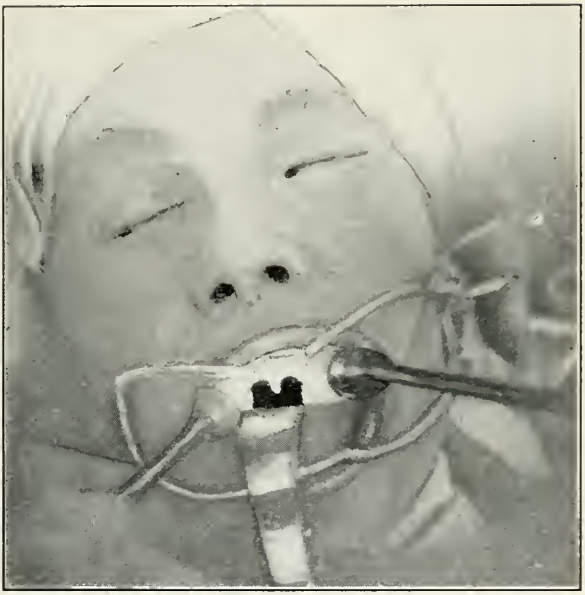


Fig. 1.—The submerged tonsils; Jennings mouth gag; electric illumination thrown from the angles of the mouth

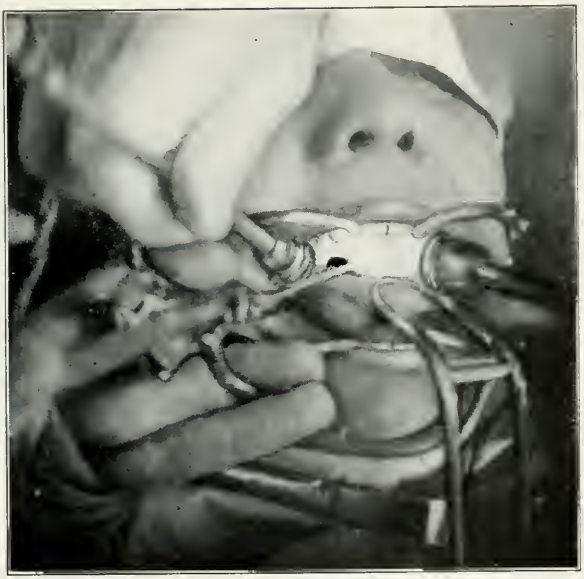


Fig. 2.—Left tonsil raised out of its bed (with the Beck instrument) before engaging; showing also the Titus tongue depressor.

Fig. 3.—Right tonsil engaged in the Beck instrument

Fig. 4.—Both tonsils engaged in the Beck instruments

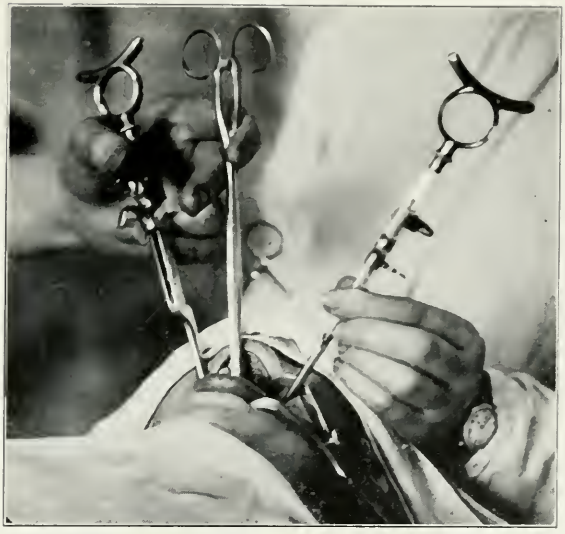


Fig. 5.—Both tonsils engaged, tenaculum applied.

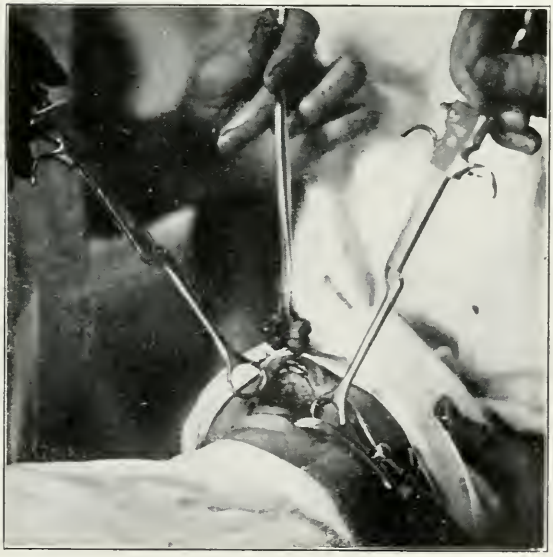


Fig. 6.—The tenaculum with enucleated tonsils

hart, Jennings, Whitehead, or Murdoch mouth gag (depending on the formation or condition of the teeth) is introduced, opening the mouth to its full capacity, and is held in place by the anesthetist; the chin slightly elevated to allow free breathing. The Titus suction tongue depressor is then introduced and the throat cleared of mucus. The advantage of this instrument is that it avoids injury to the mucous membranes and dispenses with unnecessary bleeding caused by the use of gauze or cotton sponges.

With the right hand a Beck snare is introduced from the left angle of the mouth, the loop passing posterior to the right tonsil. The tonsil is then forced through the loop, the catch is sprung and the snare wire drawn tight and locked. Two or three turns of the screw insure the engagement of the snare, which is now handed over to the assistant. In like manner the left tonsil is forced through the loops of a second Beck instrument and engaged, the manipulation of the instrument being with the left hand. This snare is also placed in the hand of the assistant.

The assistant then gently raises the snares, thus approximating the tonsils in the median line; the operator applies a tenaculum to both tonsils at the same time. The assistant takes one instrument, the operator the other and slowly the tonsils are enucleated, the assistant removing the tenaculum holding both tonsils.

What little bleeding follows the operation is usually controlled by gentle pressure, by placing vaseline coated gauze pledgets, held on sponge sticks, between the pillars. Hemorrhage seldom occurs if this method is adhered to.

This operation has many advantages. Aside from the small quantity of blood lost there is no method that leaves so little traumatism; the uvula is never sacrificed or bruised and the pillars are left intact. Pain following the operation is minimized, the slough quickly disappears and healing occurs in a very few days, leaving perfect anterior and posterior pillars.

The linear scar so frequently met with after tonsil operations, showing adhesion between the styloglossus and stylopharyngeus muscles is never present.

The after-treatment is of the simplest kind: children are seldom detained longer than twenty-four hours and are dismissed with the admonition to gargle or wash their mouths with cold or warm

water according to preference and advised to chew flavored gum for a few days, the threefold object being to get rid of the unpleasant after taste of anesthesia and of sloughing tissue and to exercise their jaws.

In adult patients when general anesthesia is not advisable local tonsillectomy is performed as follows:

The patient is given Magendie solution, minims vii, with atropine sulphate gr. 1/150, an hour prior to operation and kept quietly in bed until wheeled into the operating room; then seated in a chair and draped. The mucous membrane of the mouth and pharynx is painted several times with a 10 per cent. solution of cocaine and after an interval of ten minutes the tongue is depressed and a ½ per cent. solution of novocaine is injected into the anterior and posterior pillars, parallel to and ⅛ inch from the free border. After an interval of one minute and with the tongue depressor again in position, using the Robertson curved tonsil knife (sharp on both edges and dull at the point), an incision is made beginning at the lowest point of the anterior pillar and with a quick movement of the hand carrying the incision upward to the angle between the pillars then, sweeping downward behind the tonsil and between it and and the posterior pillar, using the other edge of the knife, to a point opposite the place of beginning. The left tonsil is firmly grasped with a tenaculum at the highest possible point, gentle traction made on it with the left hand, and the tonsil separated from its bed by the knife, in the right hand, making pressure first against the stylopharyngeus muscle, then the lateral constrictors and, finally, the anterior pillar. A snare is then used to free it from its lower point of attachment. The patient is quickly given an iced saline mouth wash, which is usually sufficient to control bleeding, after which the right tonsil is similarly removed.

The patients as a rule complain of little pain except during the separation of the tonsil from its bed, when traction is made upon it.

The after treatment consists of an ice bag to the angles of the jaw, the length of time suiting the patient's pleasure: a gargle of alkaline saline solution; orthoform tablets gr. v dissolved on the tongue ad libitum, and codeine gr. ¼ to control pain if present. The latter is repeated until comparative comfort is obtained.

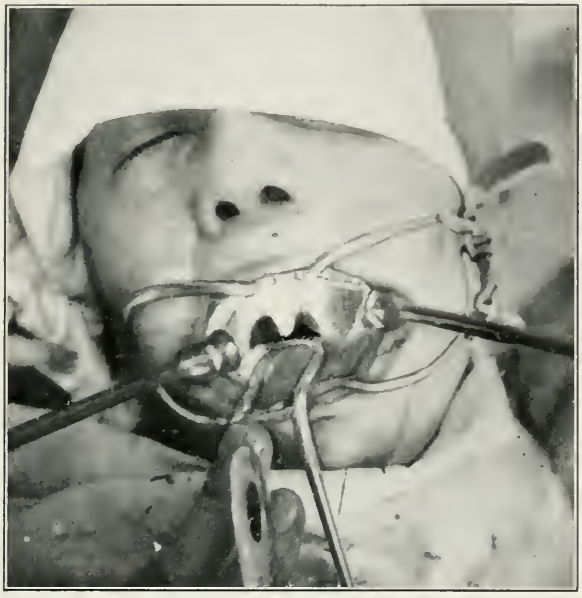


Fig. 7.—The pharynx after operation.

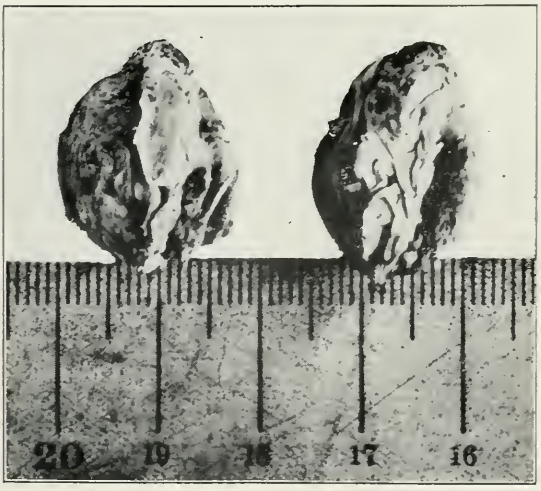


Fig. 8.—The tonsils.

The following day several local applications of glycerite of tannin are made to the raw surfaces, the slough disappearing in from two days to a week, depending on the size of the wound.

The results obtained by these methods after a year's trial are encouraging and seem to warrant a continuance of them.

I wish to express here my thanks to Dr. Arthur B. Eisenbrey for the photographs and to Dr. Thomas R. French for his kindness in retouching same.

PITYRIASIS ROSEA OF THE PALM

WILLIAM BAYARD LONG, M.D.

The following case report is believed to be worthy of record, partly because of the rarity of the appearance of the lesion in this location, but chiefly because it calls attention to the difficulty in distinguishing the eruption as it appeared in this case from that of a palmar syphilide of the late secondary type.

The eruption of pityriasis rosea occurs most frequently on the trunk, the so-called "vest area" being usually the site of predilection. Quite frequently the extremities are involved, but seldom below the level of the wrists, on the upper extremity, and of the lower third of the thigh on the lower extremity. Only very occasionally is the face involved, the eruption rarely spreading above the level of the lower border of the inferior maxilla. The rarest sites of involvement are the hands, the feet, and the penis. Regarding the three last mentioned, it may be stated that the standard dermatological works, either dismiss the subject without mention or state definitely that the eruption does not occur in these locations. Cases involving the hands and feet have, however, been reported by competent observers. Moingard reports an instance where the eruption involved the backs of the hands and feet. Stowers describes a case with involvement of the face, hands and feet. Colcott Fox described a probable pityriasis rosea with the eruption involving the fingers and toes, and palms and soles: he also cities another case with definite involvement of the dorsa of the hands and feet.

In the present case, difficulty in diagnosis arose because of facts which will, perhaps, be best brought out by citing the case history. The patient, male, an Italian, age 29, barber, appeared at the dispensary on January 12, 1915. He volunteered the information that he had contracted syphilis three years previously and that he feared he had a return of the trouble. He described a sore

on the penis, which he thought had appeared about a week after exposure, and which lasted about six weeks. He had been treated locally at this time, and also internally for a period of a few months at the longest. The examination revealed a typical eruption of pityriasis rosea, confined to the trunk, with the exception of a few patches on the upper and inner surfaces of both thighs. On the dorsum of the penis, just back of the coronal sulcus, was seen a smooth, white, dense cicatrix, with a serrated margin, the remains of the "chancre" above mentioned. The inguinal and posterior auricular glands were enlarged. No others were palpable. The Wassermann reaction was negative.

The patient was told that his eruption was not syphilitic in origin, and that he probably had not contracted syphilis. In drawing such a conclusion, the following facts were considered of importance, in the order to be mentioned. First, the character of the eruption, which was a typical text-book picture, even to the history of the appearance of the primary plaque, followed some time later by the more generalized eruption. Second, the Wassermann reaction; for while a negative reaction does not guarantee an absence of lues, yet in a case such as this of only three and a half years duration, with practically no treatment (none at all for three years), a carefully done Wassermann reaction, which is negative, speaks very strongly against such a probability. And, lastly, the history. A sore appearing one week after exposure, and not followed by any subsequent complications, such as skin eruptions, sore throat, etc., may be lues, but, admitting that such histories are notoriously unreliable, such evidence as is presented is negative, rather than positive. The eruption at this time appeared to be undergoing the ordinary slow involution, typical of such cases.

On March 2, 1915, the patient reappeared at the dispensary, afer an absence of three weeks, with fresh lesions on both upper arms, the right forearm, and the right and left palms. Those on the arms and forearms, resembled in every particular the lesions previously present on the trunk, and were recognizd as an extension of the same process. On the right palm there were five erosions, each about the size of a ten cent piece, dirty gray in color, roughly circular in outline, arranged annularly, and with indistinct, slightly raised, non-indurated borders. The centers were

wrinkled, and covered with fine, grayish white, moderately adherent scales. On the left palm was a single erosion, unlike those of the right palm, in that it was beefy red in color, sharp in outline, with no scales and a smooth floor. There were no subjective symptoms from the lesions of either palm.

Such a state of affairs opened up two possibilities. Either the palmar lesions were very unusual manifestations of the pityriasis rosea, or they were papulosquamous syphilodermata of the late secondary period, such as occur independently of any more generalized eruption. They did not resemble at all the more superficial palmar lesions, which occur as part of a relapsing, generalized secondary eruption.

In order to clear the matter up, the patient was given, on March 18, 1915, an intravenous injection of salvarsan (0.3). It was felt that this would serve two ends; first, if the eruption was luetic, a very appreciable change in the lesions could confidently be expected in the course of a few days; second, it would serve as a provocative injection for further Wassermann examinations. The salvarsan, however, exerted no appreciable effect on the eruption, which, together with the lesions on the trunk, went on to slow but steady involution without any further treatment, local or otherwise. On April 16, 1915, when last the patient was seen, scarcely a trace of the entire eruption was visible. Following the injection of salvarsan, a specimen of blood was taken on alternate days, for a period of two weeks, fourteen specimens in all being examined. All the specimens showed a frankly negative Wassermann reaction.

A CASE OF DIFFUSE SCLERODERMA

William Bayard Long, M.D.

Diffuse scleroderma, as the name implies, is a disease of the skin, the essential symptoms of which are a gradual thickening and hardening of the integument, depending on a hypertrophy of the connective tissue of the derma or true skin with a consequent obliteration of the vessels and glands and a pressure atrophy of the epidermis or horny layer. It is of very infrequent occurrence, a fact which warrants this report.

The disease occurs usually in adult life, and the onset is gradual. It affects chiefly the extremities, head, and upper half of the body. The affected skin becomes thickened, smooth, and waxy, with some loss of surface temperature and a diminution of glandular secretion. There may be a preliminary edematous phase when the skin pits on pressure, later, as the purely hypertrophic phase makes its appearance, this pitting disappears and the skin becomes firm and board-like and limits motion when it occurs in the neighborhood of joints. The course of the disease is chronically progressive and there may finally ensue an atrophic phase with shrinkage and contraction of the connective tissue resulting in deformity (sclerodactylia). All therapy has proved unavailing although improvement has been noticed after the administration of thyroid extract. The disease is progressive, lasting for years, and death is caused usually by asthenia plus some intercurrent disease.

The case forming the basis of this report was admitted to the hospital on July 1, 1915, in the service of Dr. Frissell, who has kindly furnished the notes of the very complete physical and laboratory examinations which follow.

The patient is a Russian, age 36 years; a plumber by occupation. *Chief complaint:* Weakness and stiffness of extremities. *Past history:* Negative, except for occasional digestive upsets asso-

ciated with headache and relieved by self-induced vomiting. *Family history:* Father, mother, two brothers, and five sisters living and well. Patient is the eighth child.

Present history: Patient dates the onset of his present trouble from April, 1913, when he entered Mt. Sinai Hospital complaining of weakness of both legs. At this time, both legs were swollen, with some stiffness. He remained in the hospital four weeks, and left at his own request unimproved. The swelling of legs gradually subsided, but a slight stiffness, about such as he has now, has remained since that time. In October, 1914, he noticed that the backs of both hands and the lower part of the forearm were becoming swollen and stiff. The swelling here gradually subsided, but the stiffness remained and is increasing. This is accompanied by a feeling of general weakness and he becomes easily fatigued. His sexual desire and power are sluggish and his memory is slightly impaired.

Physical examination: Patient was a poorly nourished adult male about 35 years old. The examination was negative, except as regards the skin and extremities. The patient appeared to have lost some weight, and the skin over nearly the whole body was tightly drawn and in general showed hyperpigmentation. The uninvolved portions of the integument were the lateral aspects of the arms and chest, the upper arms, the neck, the inner aspects of the thighs. The skin felt tough and thickened, and could not be picked up easily. All bony landmarks were very prominent. The skin of the face was firm and tightly drawn, making the mala bones prominent and giving the patient a mask-like expression. The normal markings of the skin were almost obliterated.

The wrists and hands showed the process in its most advanced form. Motion was limited to very slight flexion of wrists and fingers; passive motion was limited by stiffness. There was no tenderness. The skin felt much thickened, and was held tightly to underlying structures. There was no pitting on pressure, and no disturbance of sensation. The hands felt cool and waxy to the touch. The bones of the hands and fingers felt thickened, which was probably accounted for by the sclerotic condition of the underlying muscles. The *x*-ray findings were negative.

Other laboratory tests carried out in great profusion and detail were completely negative as were special examinations of the eyes, nose, throat, teeth, central nervous system, etc.

Pathological report. "A small piece of skin was removed from the back of the wrist for examination. It showed a complete fibrosis and collagenous hypertrophy of the derma, and hypoderm with atrophy of the epidermis and practically complete disappearance of the glandular structures, obliteration of the vessels, and a replacement by connective tissue of all fat elements."

The earliest changes are generally recognized to be a hyperplasia and hypertrophy of the connective tissue elements with a perivascular infiltration of cells of unknown origin, and a gradually progressive obliterating arteritis due to pressure of the connective tissue elements. Some, however, believe that the primary change is a vascular one, and that the connective tissue alterations are dependent upon changed conditions of vascularity. Whichever may be the true explanation of the sequence of the pathological findings, it would seem probable that the primary etiological factor resides in a change in the central nervous system, or in some disarrangement of function of one or more of the endocrine glands, although there is no positive evidence at present to support either assumption. Changes in the central nervous system at autopsy have been reported, but they have been so varied as to suggest coincidence rather than any real factor. All of the ductless glands have been investigated, at one time or another, with negative results, except perhaps as regards the thyroid, which seems to be involved more frequently than any of the others; whether this is the result of coincidence or not, has not at present been determined.

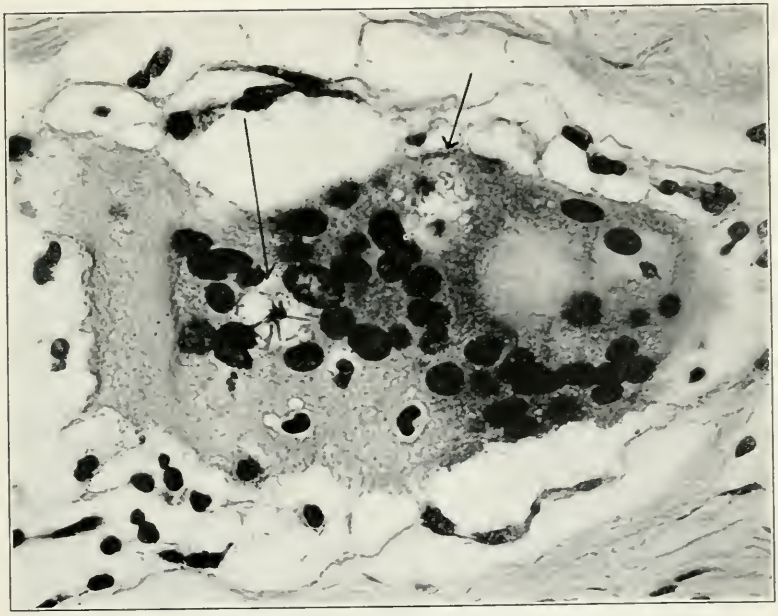
FOREIGN BODIES IN GIANT CELLS

F. C. WOOD, M. D.

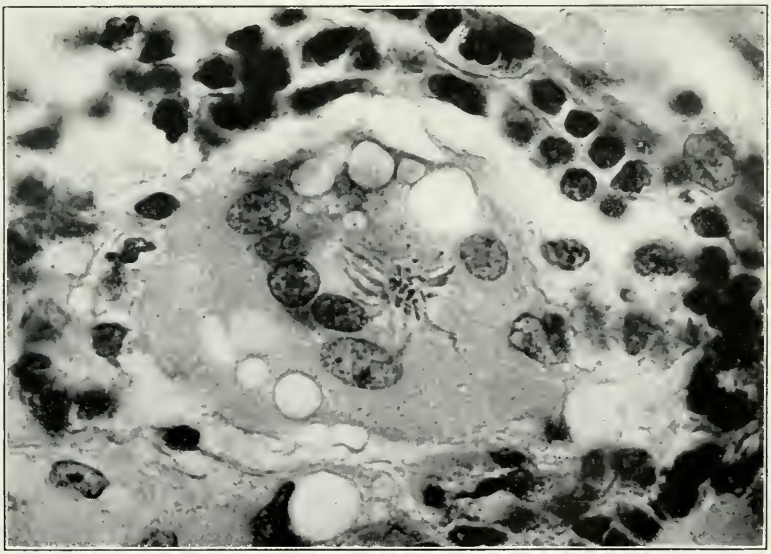
The patient from whom the material to be shown was removed was a woman (C. B., St. Luke's Path. No. 11022), twenty-six years of age, who about a year previous had had a paraffin injection in the neck for cosmetic purposes. Owing to the considerable hyperplasia which resulted from the injection, it was necessary to remove the mass surgically.

The tissues showed the usual morphological appearance found after such injections, consisting in a very considerable inflammatory reaction of a chronic nature with many lymphocytes and plasma cells lying in a meshwork of granulation tissue. Scattered throughout this granulation tissue were numerous giant cells, some surrounding globules of the paraffin, and others not showing any included structure, while a considerable proportion contained stellate bodies which occupied the center of the cells. These bodies consisted of a central mass, 2 to 3μ in diameter, from which sprang curved spicules, fifteen to twenty in number. The diameter of the whole body was 20 to 25μ. These bodies stained fairly well with hematoxylin and somewhat more deeply with Weigert's elastic tissue stain. In the writer's opinion, they probably represent condensed protoplasmic shells about crystals of paraffin, or possibly fatty acids. Mallory and others consider that they are fibrinoid in nature.

The literature concerning these structures, which have, of course, only an academic interest, is not very extensive. The first report was that of Ris, who described similar giant cells in an omental cyst removed from the peritoneal cavity of a man, fifty-one years old. The cyst was about 7 cm. in diameter, with a wall 4



Stellate Bodies in Giant Cells. ×750.



Stellate Bodies in Giant Cells. ×750

mm. in thickness. The lining of the cyst was composed of granulation tissue with many cholesterin crystal giant cells and also giant cells containing the peculiar stellate structures under discussion. Ribbert, who saw the preparations, thought that they were imperfectly developed portions of moulds (*Schimmelpilze*). These stellate bodies did not stain with the ordinary nuclear stains nor with Weigert's fibrin stain. They measured about 15 to 25 μ in diameter and were composed of curved needles radiating from a central homogeneous area. About one-third of the giant cells contained these bodies.

The next report was that of Wolbach, who stated that in 900 autopsies he had found five cases in which giant cells contained small radiating inclusions. In the first case the structures were present in the lungs; in the second, in the lymph nodes; in the third, in the lungs and lymph nodes; in the fourth, in the spleen, liver, and lymph nodes; and in the fifth, in the lung, spleen, and liver. In one case they were found in the peripheral sinuses of the lymph nodes. The smaller forms were often included in endothelial leucocytes and occasionally within endothelial cells which were attached to the walls of the lymph spaces. Wolbach called attention to the fact that these inclusions were not parasites though they resembled structures occasionally encountered in experimental lesions produced by certain fungi. He held that it was impossible that they should be artefacts. For the material which he studied the best stain was found to be Mallory's phosphotungstic acid hematein; Gram's stain colored the central body and the spines a deep blue, the intervening material taking the counterstain; and with Mallory's eosin-methylene blue the spines and central body were stained a deep purple and the intervening portion a pink color. The bodies were not soluble in caustic potash or in hydrochloric acid, and were not stained by silver nitrate in frozen sections of formalin material. Scharlach R and osmic acid did not stain them nor did iron hematoxylin or Weigert's elastic tissue stain. The iron reaction was negative.

Vogel described similar bodies in giant cells in a case of obliterative capillary bronchitis in a child eleven years old. The giant cells were found in the connective tissue replacing the bronchi and the perivascular connective tissue. They contained one to

three star-like bodies which suggested crystals; took a rather faint stain with hematoxylin, and colored best with Weigert's stain for elastic fibers. Fat, fatty acids, and iron could not be demonstrated in the giant cells. Vogel thought that these bodies were probably not altered elastic tissue, but he could give no suggestion as to their nature. He referred to articles by Ssudakewitsch and Rona, but the structures figured by these two authors do not in the least resemble the stellate bodies under discussion.

Iwanzoff found similar structures in a myomatous uterus removed from a woman, fifty years old. In this myoma, which showed a considerable amount of hyaline degeneration, there were areas of lymphoid infiltration containing irregular giant cells. In some cases these giant cells were so abundant that they formed a large part of the nodule. There was nothing peculiar about the giant cells, except that when stained with Weigert's elastic stain they showed peculiar radiating structures previously described by Wolbach. They were not bacterial in nature and did not stain except with Weigert's stain. The writer discusses the question of whether these are phagocyted elastic tissue fibers, such as are frequently seen in tuberculosis of the skin, whether they are parasitic inclusions, or whether they are astrospheres. He referred to a paper by Wakabayashi in which the astrospheres in tuberculous and other giant cells are described, and thought that these bodies closely resembled the structures which he had reported.

Mallory in his text-book shows a giant cell (Fig. 120, page 207) from a case of leprosy, which contains a spiculated body of the type under discussion. He gives no details except the legend under the cut. In another cut he shows similar spiculated bodies (Fig. 465, page 613), the text referring to these reading as follows:

"Spiculated bodies have been found in the spleen in a few instances inclosed in endothelial leukocytes and giant-cells. They may be quite numerous and occur in foci or diffusely scattered. The lesion resembles miliary tuberculosis, but no necrosis is produced. The bodies seem chemically to be of a fibrinoid character. They are evidently not the cause of the lesion, which is probably of infectious origin, but a secondary formation."

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BLOOD TRANSFUSION AND REGENERATION IN PERNICIOUS ANEMIA*

KARL M. VOGEL, M.D., and U. F. MCCURDY, M.D.

A feature of much interest in the observation of cases of pernicious anemia is the behavior of the reticulated cells. In six cases of pernicious anemia, four of them treated by transfusion, which are here reported, this element of the blood-picture was studied with special care, and from these as well as from earlier cases of transfusion done in St. Luke's Hospital, certain conclusions are drawn in regard to the value of vital staining in cases of severe anemia, and concerning the subject of transfusion in general.

Ever since the discovery of the fact that by treating fresh, unfixed blood with solutions of various dyes, such as brilliant cresyl blue, polychrome methylene blue, neutral red, pyronin methylene green, etc., a curious and otherwise invisible structure could be demonstrated in the red cells, there has been much speculation as to its significance. Ehrlich, Pappenheim and Horsley were among the first to describe the phenomenon, but nearly all the subsequent studies have come from French and Italian laboratories (Hertz,[1] Ferrata and Boselli[2]). In suitably treated preparations this so-called granulo-filamentous or reticulo-filamentous substance appears in the form of coarse granular particles which are sometimes discrete, but more often occur in threads which frequently are woven into skeins or wreaths of great complexity and fill a considerable portion of the cells. In the blood of infants these reticulations are found in from 5 to 10 per cent. of the erythrocytes and in normal adult blood in from 0.5 to 2 per cent. In severe anemias, however, their number is much increased, running as high as 18 to 20 per cent., while in hemolytic jaundice, which is the condition par excellence for their prevalence, they may occur in still greater proportions.

* Reprinted from *The Archives of Internal Medicine*, December, 1913.

Without taking the space at this time to enter into a discussion of the arguments pro and con of the various writers who have studied the matter, we may summarize briefly the views to which our own observations and a consideration of the literature have led us, by saying that the granulo-filamentous substance is not derived from the nucleus, is different from polychromatophilia and from the basophilic stippling seen in fixed preparations stained with panchromatic dyes, is not a preformed structure, but is a precipitation product of the stain, and is an evidence of youth in the cells and not of degeneration. In conditions in which a severe drain on the erythrocytes is being sustained by a well-functionating bone-marrow, large numbers of reticulated cells are found, whereas in aplastic cases they may be diminished almost to the point of absence, that is, in a manner somewhat comparable to the behavior of the erythroblasts the reticulated cells afford a direct insight into the hematopoietic activities of the bone-marrow. For clinical purposes they form a more convenient measure of this function than do the nucleated cells, as their percentage relations to the erythrocytes can be more easily and accurately determined, and their enumeration is to be urged as a part of the study of the blood in all cases of severe anemia. The relationship between the presence of the reticulated cells in the peripheral blood and the hemopoietic activity of the marrow was well illustrated in the case of a young man suffering from purpura hemorrhagica seen since the above was written. In spite of three transfusions of about 700 c.c. each, done by direct intravenous injection, the red cells rapidly became reduced to 1,500,000, hemoglobin 25 per cent., while the leukocytes were 2,800, polynuclears 12 per cent. and lymphocytes 88 per cent., death resulting within two weeks of the onset of the symptoms. A highly significant feature was the total absence of reticulated cells, showing the complete inability of the bone-marrow to respond to stimulation.

The following technic for vital staining may be used:

TECHNIC FOR VITAL STAINING

Saturate 0.85 per cent. salt solution with brilliant cresyl blue. Filter this through a double paper to take out the excess of dye substance and to prevent precipitation on the slide. It is better to centrifugalize the stain before using it, in order to be sure that no undissolved particles remain in suspension. If any precipitate is present in the stain it will later be thrown

down on centrifugalizing the mixed blood and stain and will cause confusion in counting the reticulated cells.

The following should be freshly prepared before use:

Saturated solution of brilliant cresyl blue in 0.85 per cent. salt solution.

Salt solution, 0.85 per cent. .....aa. 5 c.c.

Sodium oxalate solution, 2 per cent. .....2 c.c.

Add the oxalate to the salt solution, then mix with the stain and filter.

Puncture the finger so as to get a free flow of blood, draw a good-sized drop into a red cell counting pipet, and using the stain as a diluent fill the diluting chamber. After thorough mixing, allow this to stand for ten minutes, then blow the contents of the mixing-chamber into a centrifuge tube and centrifugalize. Draw off the staining fluid with a capillary pipet, until only the sediment of cells remains. Draw the cells from the bottom of the centrifuge tube into a capillary pipet, then place a drop of these cells on the end of a clean slide which has been slightly warmed in a flame, and spread as in making ordinary blood-smears. The preparation will remain permanent indefinitely if mounted in neutral balsam or dammar and not exposed to strong daylight.

In counting the cells the use of an Ehrlich eyepiece is of great assistance. A satisfactory substitute may be improvised, however, by cutting a hole about 8 mm. square in a disk of blackened cardboard and dropping this disk over the diaphragm of the ordinary eyepiece.

In the following cases of pernicious anemia, systematic determinations of the reticulated cells were made in connection with the study of the blood-picture by the usual methods, and as will be seen from the charts and the remarks in the clinical histories, an interesting relationship can be derived between the fluctuations of the red blood-cells and the curve of the reticulated cells.

The detailed histories of the cases are as follows:

REPORT OF CASES

CASE 1.—*History* —M. M., a school teacher, aged 45, was admitted to the service of Dr. H. S. Patterson, Nov. 11, 1912. The chief complaints of the patient on admission were a feeling of weakness, dyspnea, palpitation of the heart and vomiting. About Jan. 1, 1912, she first noticed a feeling of languor and loss of appetite. In a short time the color of her skin and eyes alarmed her and she consulted a physician who treated her for "jaundice and weak kidneys." She was put to bed in February and remained there until April. For about a month she felt well and strong. In May she had palpitation of the heart on the least excitement and suffered from attacks of vertigo and morning headaches. Occasionally she had morning vomiting. From June to September she suffered from irregular short periods of colitis. She had been in bed for three months prior to admission, the most prominent symptoms being weakness, vomiting and anorexia.

Examination.—The patient is poorly nourished and acutely ill. Her complexion is pale and of a lemon-yellow tint. The sclerae have an icteroid tinge. The teeth and mouth are in good condition. The lungs are clear, the heart is of normal outline, the sounds are of good quality and are regular. There is a booming systolic murmur all over the precordium, which is loudest at the apex. There is no accentuation of the pulmonic or aortic sounds. The knee-jerks are absent. The blood-picture on admission shows red blood-cells 1,160,000; hemoglobin, 22 per cent.; white blood-cells, 5,000;

polymorphonuclear leukocytes, 75 per cent.; lymphocytes, 25 per cent. The erythrocytes show marked poikilocytosis, anisocytosis, polychromatophilia and an occasional normoblast, but no megaloblasts. The reticulated erythrocytes are only 0.5 per cent. of the total, showing that no active regeneration is going on. The Wassermann reaction is negative. The analysis of the stomach contents after a test-meal shows absence of free hydrochloric acid and a very low total acidity.

For two weeks the patient continued to grow worse, and the cell-count fell rapidly.

Treatment and Course.—December 5 her condition was so precarious that an immediate transfusion was decided on. A count made a few hours prior to the transfusion showed that the reticulated erythrocytes had risen to 8.2 per cent., and that there was an attempt on the part of the bone-marrow to produce new cells. The majority of the reticulated cells were macrocytes. The patient received only a small amount of blood at the time of transfusion, as the accompanying counts show. After remaining for from four to five days in a semicomatose condition, she began to improve rapidly. About a week after the transfusion sodium cacodylate grains ¾ daily subcutaneously was started. The rapid improvement which followed the transfusion seems to imply that the stimulating influence of the presence of the donor's blood was sufficient to arouse the patient's marrow into renewed activity. It also suggests that since with the small amount of blood given in this case a favorable result was obtained, transfusion of a large amount of blood is by no means always necessary. The patient has improved steadily up to date. Although the blood-picture has returned practically to normal, the gastric achylia is still as pronounced as at first. It was subsequently learned that about six weeks after leaving the hospital this patient underwent a relapse. She was transfused twice more, but with little benefit, and early in July she died. A very interesting point is the sharp rise in the percentage of the reticulated cells preceding the rise in the erythrocyte count and the gradual fall of the

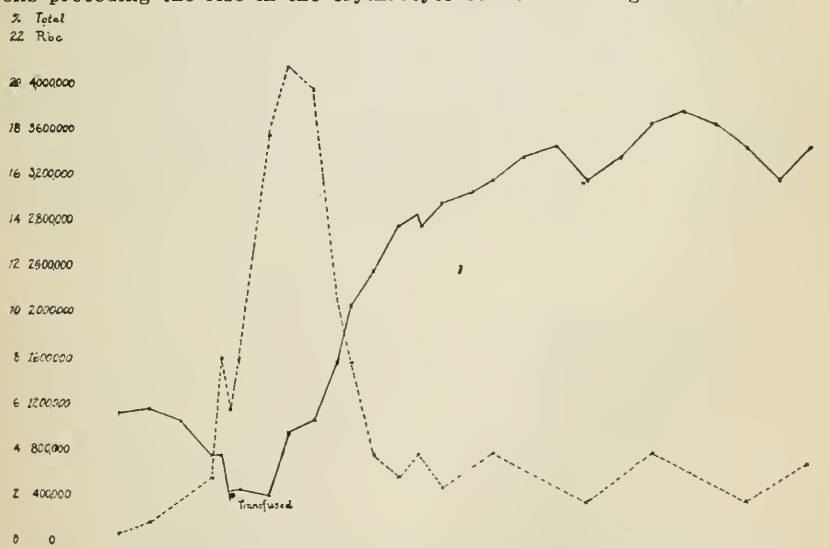


Chart 1.—Blood-findings in Case 1. In this and the following charts the solid line indicates the number of total erythrocytes, and the dotted line the percentage of reticulated cells.

percentage of the same cells as the total erythrocyte count became higher. As seen by Chart 1, the reticulated cells gradually fall to the percentage found in normal blood as the total count approaches its maximum.

Case 2.—*History.*—M. L., a housewife, aged 52, was admitted to the service of Dr. S. W. Lambert, Dec. 12, 1912. Up to the time of the onset of the present illness the patient says she has been in perfect health. In February, 1912, after a short period of failing appetite, her attention was attracted to the sallowness of the skin and the yellowish tinge of her eyes. During the weeks following a general sense of lassitude developed, and with it attacks of vomiting, at irregular intervals. During the last six months she had noticed that the vomiting is less when she is quiet in bed than when up and about her work. In April she was in bed for one month for "nervous prostration." Subsequent to the rest she was able to resume her household duties with little physical discomfort besides shortness of breath and occasionally numbness of the hands and feet. Six weeks before admission her weakness forced her to give up all work and go to bed. She has lost 37 pounds during the last year. The patient comes into the hospital "without a pain or an ache, but feeling miserably weak."

Examination.—The patient is pale, appears jaundiced and looks chronically ill. Her sclerae are distinctly yellow, and her left pupil is larger than the right. Her speech is slow and drawling. The teeth are in poor condition, with many missing. The lungs are clear. The heart is normal in size, but shows a faint systolic murmur, which can be heard all over the precordium, loudest at the apex and pulmonic areas, and transmitted to the back and axilla. The analysis of the fasting stomach contents and also that of the test meal shows achylia. The Wassermann reaction is doubtfully negative. The blood-picture is one of typical pernicious anemia. The morphology of the red cells shows poikilocytosis, anisocytosis, polychromatophilia and an occasional megaloblast and normoblast. The color index is 1.3.

Treatment and Course.—The patient could not be induced to take sufficient nourishment, declined steadily, and December 26 was transfused. The transfusion was very successful, both as regards the patient's immediate condition and the microscopical findings. The value of the microscope as a control in the operating-room during transfusion was demonstrated in this case. At the end of fifty minutes, when the surgeon was satisfied that the patient had received a large amount of blood, a red-cell count and hemoglobin determination showed that the amount received was almost negligible. A new anastomosis was made and in forty minutes the patient received a quantity sufficient to raise the total cell-count 1,000,000 per cubic centimeter. For a few days the patient was appreciably brighter, but the improvement

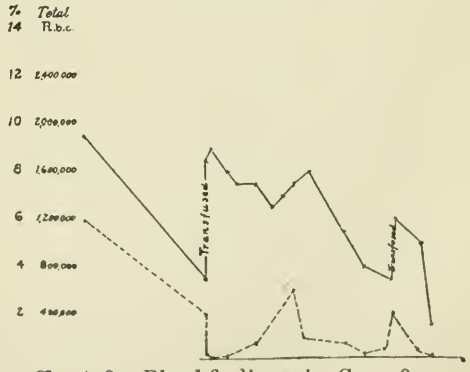


Chart 2.—Blood-findings in Case 2.

was not long continued. For a month the total cell-count varied little. The vital staining of the erythrocytes showed plainly the presence of the donor's cells in the smears, for from the first these did not take the stain as well as those of the recipient. From day to day they could be seen to have lost their hemoglobin to a greater and greater degree. After about two weeks they existed as shadow outlines of the original cells. At the end of three weeks a considerable number of these shadow cells were still present. There was no active regeneration on the part of the patient's marrow as far as could be determined by the blood-picture. The reticulated cells never reached more than 3 per cent. of the total (Chart 2) and megaloblasts and normoblasts were rarely seen. January 30 a second transfusion was done. While this time only one-half as many cells were given, the patient seemed toxic from immediately after the operation to the time of her death one week later.

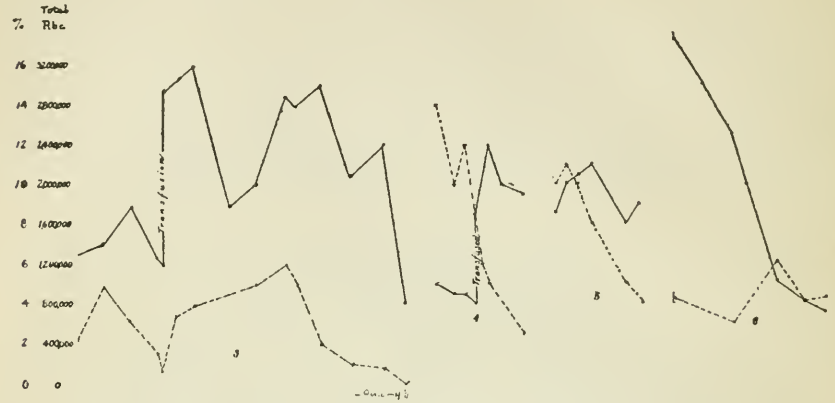
Case 3.—*History.* —Dr. D., a Cuban physician aged 58, was admitted to the service of Dr. A. W. Hollis. In 1898 the patient was "executed" shortly before the beginning of the Spanish-American War. One bullet pierced the right shoulder and another passed through the body just above the trochanters as he fell. He was left for dead, but was later taken to the hospital in Havana, where he received treatment. Since that time he has had occasional attacks of diarrhea. During the last five years he has suffered from colitis, at irregular intervals and for periods of five to ten days, but has never regarded these as serious. Two years ago he was told that he had severe anemia. He was not incapacitated for work, but during the last few months has lost much strength and weight. For the last two months the attacks of colitis have recurred with increasing frequency until the last two weeks, since when he has had from eight to ten stools a day, accompanied by much tenesmus. He vomits frequently and has morning headaches. His tendency to dyspnea and vertigo has necessitated his remaining in bed for the last two weeks.

Examination.—The patient is poorly nourished and shows signs of emaciation. The sclerae are yellow; the skin is pigmented. The mouth and teeth are in good condition. The lungs are clear. The heart is not enlarged, the sounds are weak and no murmurs are present. The abdomen shows general tenderness, especially over the region of the colon. The Wassermann reaction is negative, as is the blood-culture.. The stomach contents show low free hydrochloric acid and low total acidity. The stools examined on several occasions always give positive tests for free blood. No parasites or ova are found.

Treatment and Course. —On admission the patient was acutely ill and much emaciated from his colitis. The colitis could not be checked after about three weeks' time; the weakness and vertigo were still prominent symptoms and transfusion was decided on.

An artery-to-vein anastomosis was made and the blood allowed to flow for forty-five minutes. The total cell-count in the recipient was increased from 1,200,000 to 3,000,000 erythrocytes per cubic millimeter. The blood-picture before the transfusion had shown extreme poikilocytosis, anisocytosis, polychromatophilia and stippling of the erythrocytes. The immediate effect of the transfusion was apparent in the subsidence of the patient's symptoms with the exception of the colitis. This increased in severity for three or four days and then was checked. Much improvement followed for a month, the patient keeping the donor's cells and regenerating his own rapidly enough to keep the cell-count over 2,500,000. The "shadow cells" of the donor persisted for about three weeks in the blood-picture. The reticulated erythrocytes never amounted to over 6 per cent. of the total count (Chart 3). March 5 the patient on his own initiative was given salvarsan intravenously. The colitis at once again became severe, but in spite of this there was temporarily

sufficient improvement to permit the patient to leave his bed. He insisted on a second dose of the salvarsan, but immediately after this the cell-count dropped and death followed in five days.



Charts 3, 4, 5 and 6.—Blood-findings in Cases 3, 4, 5 and 6.

Case 4.—*History.* K. L., a German housewife, aged 46, admitted to the service of Dr. S. W. Lambert, May 2, 1913. The patient complains of weakness and hemorrhage on entering the hospital. Her family and past histories are negative. She dates the onset of her present illness from the time about two years ago, when she suffered from "fever and chills" for several weeks. Since then she thinks that she has been losing weight and has gradually become weaker. About two months ago, at her regular menstrual period, the patient had a hemorrhage, during which she thinks she lost a large amount of blood. One month ago she discovered that she was unable to climb the stairs to her apartment because of weakness, shortness of breath and palpitation of the heart. During the last two weeks she has had three attacks of nosebleed. Six weeks ago the patient took some medicine which upset her stomach, and she has vomited almost daily since. She has numbness of the hands and feet. She is sure that she has lost 25 pounds in weight in the last year.

Examination.—The patient is a poorly nourished woman of middle life, who appears chronically ill. Her skin is of a deep lemon color and the mucous membranes are very pale. She is weak and prostrated. The sclerae have a slight icteroid tint. The mouth is in poor condition, containing many carious teeth. The tongue is moist, and heavily coated. The lungs are clear. The heart is normal in outline. The sounds at the apex are regular and of fair muscular quality. The first sound is accompanied by a loud blowing systolic murmur, heard all over the precordium and transmitted to the axilla. The pulse suggests the Corrigan type. The patient's Wassermann is doubtfully positive and her blood-culture is negative. No gastric analysis was made because of her inability to retain food.

Treatment and Course.—Although there was no apparent change in the blood-picture during the first week of the patient's stay in the hospital, she grew rapidly weaker because of the inability to retain food. Over two-thirds of the nutrient enemata were expelled soon after they were given. May 10 she was transfused; the total cell-count after transfusion was 900,000 per cubic millimeter more than before. Before the transfusion the blood-picture had been one of active regeneration. The reticulated erythrocytes varied from 10 to 14 per cent. (Chart 4), and together with the presence of many

normoblasts and megaloblasts showed that the bone-marrow was still actively functioning. The effects of the transfusion were transient. The following day found her in a stuporous condition, from which she did not rally. On May 19 she went into delirium, which was followed by coma and death in a few hours. As in the other cases the cells of the donor could after two or three days be distinguished from those of the recipient, and they persisted up to the time of the patient's death. In this instance the patient received what may have been an overwhelmingly large dose of blood, and it is possible that a smaller transfusion might have been more advantageous.

CASE 5.—*History.*—Mrs. L. S., a Swiss housewife, aged 42, admitted to the service of Dr. S. W. Lambert, April 16, 1913, gives as her chief complaints general weakness and epistaxis. Eight years ago she was in the hospital complaining of a "goiter." After a few weeks in bed she was treated from the outpatient department of the hospital with "injections." She says that she then had "nervousness and a fluttering heart." These symptoms gradually disappeared and she was well until April, 1909. At that time she was treated in the hospital for pernicious anemia and was discharged with the diagnosis: pernicious anemia, acute thyroidism, chronic endocarditis. She was in the wards again in May, 1910, and April, 1911. Four weeks ago she was taken suddenly with a chill and fever. The doctor told her that she had "grip." When she attempted the resumption of her housework she found she did not have enough strength. Her face became puffy and her feet began to swell. She has been out of bed but one day since, and thinks that she has lost 12 pounds in the last month. The shortness of breath and palpitation of the heart have been growing worse. She has had several severe nose-bleeds in the last few days.

Examination.—The patient is a fairly nourished woman who appears chronically ill. The skin has an icteroid tint, and the face is slightly edematous. The sclerae are bluish-yellow. The teeth are carious and the mouth poorly kept. The lungs are clear. The outline of the heart is normal. Typical signs of mitral stenosis and regurgitation are present. The right kidney is palpable on deep inspiration. Examination of a test-meal shows the absence of free hydrochloric acid, and a total acidity of 10; no blood or lactic acid. The Wassermann reaction is negative, as is the blood-culture.

This patient remained in the hospital for a little over two weeks. She was apparently comfortable and wanted to return to her own home and resume her housework. Although the reticulated erythrocytes were present in such large percentages and indicated an active marrow, there were surprisingly few nucleated red cells. The erythrocytic picture was typically that of pernicious anemia (Chart 5). The constancy of the red blood-cell count showed that the regeneration was taking care of the cell destruction. The resistance of the red blood-cells to hypotonic salt solutions was increased, for hemolysis did not begin until a dilution of 0.38 per cent. was reached, and to cause complete hemolysis a solution of 0.32 per cent. was required.

CASE 6.—*History.* Miss C., a nursemaid, aged 43, was admitted to the service of Dr. S. W. Lambert. Beginning in September, 1911, the patient had a severe attack of diarrhea, which lasted six weeks. She had as many as fifteen movements a day. The stools contained both blood and mucus. Immediately following this she had palpitation of the heart on exertion, and was very nervous and easily excited. She was admitted to the hospital April 29, 1912, with a swelling on the front of the neck. She complained of loss of weight in spite of her good appetite. She also had marked polyuria. The superior thyroid arteries were ligatured, and while it was thought that there was improvement, the edema of the feet, nervousness, pain in the back and the weakness did not disappear. She remained in the medical wards from Sept. 16, 1912, to Dec. 21, 1912, having been transferred there from the surgical division. She was discharged on December 21 as improved. On Jan. 24, 1913, she was again admitted to the medical service, complaining of a "noise

Case	Date	Erythrocytes	Hemoglobin	Color Index	Leukocytes	Differential Count					Retic. Eryth. Per Cent.	Fragility		Coag. Time*
						P.	L.	Eo.	Ba.	M.		Hemolysis		
												Began Per Cent.	Complete Per Cent.	
1	11/11/12	1,160,000	22	0.95	5,000	75	25	..	..	..	0.5	0.40	0.34	2' 2
	11/18/12	1,200,000	20	0.83	4,600	83	16	1	..	..	1.0	....	....	2' 1
	11/25/12	1,100,000	20	0.90	4,000	78	20	1	1	..	...	....	....	....
	12/ 2/12	800,000	20	1.2	3,000	80	19	1	..	..	3.0	....	....	....
	12/ 4/12	800,000	20	1.2	....	..	.	..	..	..	8.2	....	....	....
	12/ 5/12†	400,000	12	1.5	....	..	..	..	..	..	6.0	....	....	....
		500,000	12	1.2	....	..	..	..	..	..	...	....	....	....
	12/ 6/12	500,000	15	1.5	3,000	78	20	1	1	..	7.0	....	....	....
	12/13/12	480,000	15	1.5	6,000	60	40	..	..	..	8.2	....	....	....
	12/17/12	1,000,000	20	1.0	3,000	65	33	1	..	1	8.0	....	....	....
	12/23/12	1,100,000	35	1.6	5,000	82	18	..	..	..	18.0	....	....	....
	12/28/12	1,600,000	45	1.4	....	..	..	..	..	..	21.0	....	....	....
	12/31/12	2,100,000	50	1.2	6,000	70	29	..	1	..	20.0	....	....	....
	1/ 6/13	2,400,000	50	1.0	....	..	..	..	..	..	11.0	....	....	....
	1/11/13	2,800,000	50	0.9	6,200	68	31	1	..	..	8.0	....	....	....
	1/15/13	2,900,000	65	1.1	....	..	..	..	..	..	4.0	....	....	....
	1/16/13	2,800,000	65	1.1	....	..	..	..	..	..	3.0	....	....	....
	1/20/13	3,000,000	65	1.0	....	..	..	..	..	..	4.0	....	....	....
	1/27/13	3,100,000	67	1.0	6,000	74	25	1	..	..	2.5	....	....	....
	2/ 3/13	3,200,000	67	1.0	....	..	..	..	..	..	3.0	....	....	....
	2/10/13	3,400,000	70	1.0	7,200	70	28	2	..	..	...	....	....	....
	2/17/13	3,500,000	70	1.0	....	..	..	..	..	..	1.7	....	....	....
	2/24/13	3,200,000	72	1.1	6,800	57	43	..	..	..	...	....	....	....
	3/ 3/13	3,400,000	75	1.1	4,600	62	37	1	..	..	4.0	....	...	....
	3/10/13	3,700,000	77	1.0	....									
	3/17/13	3,800,000	77	1.0	....									
	3/24/13	3,700,000	80	1.0	5,000	67	31	1	1	..	1.8	....	....	....
	3/31/13	3,500,000	77	1.1	....									
	4/ 6/13	3,200,000	75	1.1	....	..	..	..	..	..	3.5	....	....	....
	4/13/13	3,500,000	..	...	....	..	..	..	..	..	2.1	....	....	..
2	12/14/12	1,900,000	50	1.3	4,800	75	23	1	1	..	6.0	0.40	0.30	2' 26
	12/26/12†	700,000	25	1.6	3,600	68	32	..	..	..	2.0	....	....	.....
		1,700,000	45	1.2	....	..	..	..	..	..	0.8	....	....	.....
	12/27/12	1,800,000	40	1.1	2,200	58	40	1	..	1	0.25	....	....	1' 57
	12/30/12	1,600,000	32	1.0	....	..	..	..	..	..	0.28	....	....	.....
	1/ 1/13	1,500,000	30	1.0	3,000									
	1/ 3/13	1,500,000	35	1.1	3,500	76	23	1	..	..	0.76	....	....	.....
	1/ 6/13	1,300,000	35	1.3	....									
	1/ 8/13	1,400,000	35	1.2	....	..	..	..	..	..	3.0	....	....	.....
	1/10/13	1,500,000	35	1.1	....	..	..	..	..	..	1.0	....	....	.....
	1/13/13	1,600,000	32	1.0	3,600	72	28	..	..	..	0.8	....	....	.....
	1/20/13	1,100,000	22	1.0	....	.	..	..	..	..	0.37	....	....	.....
	1/24/13	800,000	20	1.2	2,300	33	64	2	1	..	0.25	....	....	.....
	1/30/13†	700,000	18	1.3	....	..	..	..	..	..	0.45	....	....	.....
		1,200,000	20	0.83	....	..	..	..	..	..	2.0	....	....	.....
	2/ 5/13	1,000,000	20	1.9	3,600	42	57	1	..	..	0.5	....	....	.....
	2/ 7/13	320,000	15	2.3	1,100	31	67	..	1	1	0.25	....	....	.....

* By the Dale and Laidlaw method. (Jour. Path. and Bacteriol., 1912, xvi, 351.)
†Transfusion. ‡Salvarsan.

BLOOD-COUNTS OF SIX PATIENTS WITH PERNICIOUS ANEMIA—Continued)

Case	Date	Erythrocytes	Hemoglobin	Color Index	Leukocytes	Differential Count					Retic. Eryth. Per Cent.	Fragility		Coag. Time*
						P.	L	Eo.	Ba.	M.		Hemolysis		
												Began Per Cent.	Complete Per Cent.	
3	1/14/13	1,380,000	35	1.3	3,300	58	42	..	..	..	2.3	0.40	0.28	2′ 20″
	1/20/13	1,480,000	35	1.2	6,300	69	31	..	..	..	5.0	....	....	.....
	1/27/13	1,800,000	40	1.1	4,900	59	40	1	..	..	3.3	....	....	.....
	2/ 3/13	1,280,000	40	1.6	3,600	46	52	1	1	..	1.6	....	....	.....
	2/ 4/13†	1,200,000	35	1.4	....	..	..	..	..	..	0.9	....	....	.....
		3,000,000	60	1.0	3,800	48	52	..	..	..	1.0	....	....	.....
	2/ 7/13	3,100,000	60	0.96	3,600	52	46	1	..	1	3.5	....	....	.....
	2/10/13	3,200,000	65	1.0	....	..	..	..	..	..	4.0	....	....	.....
	2/18/13	1,600,000	48	1.5	6,000	64	33	3	..	..	...	....	....	.....
	2/25/13	2,000,000	60	1.5	...	..	..	..	..	..	5.0	....	....	.....
	3/ 3/13	2,900,000	70	1.2	4,500	62	37	1	..	..	6.0	....	....	.....
	3/ 5/13‡	2,800,000	70	1.2	....	..	..	..	..	..	5.0	....	....	.....
	3/11/13	3,000,000	75	1.2	....	..	..	..	..	..	2.0	....	....	.....
	3/19/13	2,120,000	55	1.3	4,600	68	32	..	..	..	1.0	....	....	.....
	3/24/13‡	2,400,000	60	1.2	....	..	..	..	..	..	0.76	....	....	3′ 15″
	3/29/13	890,000	25	1.3	1,300	28	68	4	..	..	?	....	....	.....
4	4/ 2/13	1,000,000	20	0.9	10,000	48	50	2	..	..	14.0	0.40	0.30	2′ 59″
	4/ 6/13	900,000	25	1.2	5,000	50	47	3	..	..	10.0	....	....	.....
	4/ 8/13	900,000	25	1.2	9,000	65	35	..	..	..	12.0	....	....	.....
	4/10/13†	800,000	25	1.9	....									
		1,700,000	35	0.9	4,800	62	34	3	1	..	6.0	....	....	.....
	4/13/13	2,400,000	50	0.9	4,500	58	41	..	..	..	5.0	....	....	.....
	4/15/13	2,000,000	40	0.9	....									
	4/19/13	1,900,000	45	1.0	3,200	46	52	1	1	..	2.1	....	....	.....
5	4/17/13	1,760,000	40	1.1	3,000	52	47	1	..	..	10.0	0.38	0.32	2′ 10″
	4/19/13	2,050,000	55	1.3	4,600	60	38	1	1	..	11.0	....	....	.....
	4/21/13	2,100,000	55	1.3	4,200	64	34	2	..	..	10.0	....	....	.....
	4/24/13	2,200,000	55	1.2	4,500	68	32	..	..	..	8.0	....	....	.....
	5/ 2/13	1,600,000	45	1.4	....	..	..	..	..	..	5.0	....	....	.....
	5/ 5/13	1,800,000	50	1.3	4,800	70	27	1	2	..	4.0	....	....	.....
6	4/30/12	4,000,000	75	0.90	5,000	74	26	..	..	..	...	0.38	0.28	.....
	5/17/12	3,600,000	60	0.83	4,500	60	38	1	1	..	...	....	....	.....
	6/14/12	3,680,000	65	0.88	6,640	64	36	..	..	..	...	....	....	.....
	7/18/12	3,700,000	72	0.97	2,100	70	30	..	..	..	...	....	....	.....
	9/16/12	3,800,000	75	0.98	3,400	60	39	1	..	..	...	....	....	.....
	10/ 3/12	3,500,000	65	0.92	4,200	58	41	1	..	..	...	....	....	.....
	11/29/12	3,500,000	55	0.80	5,500	60	40	..	..	..	4.2	....	....	2′ 24″
	12/ 6/12	3,000,000	69	1.1	8,000	64	36	..	..	..	...	....	....	.....
	12/13/12	2,500,000	75	1.5	5,800	66	32	..	2	..	3.0	....	....	.....
	1/24/13	1,000,000	20	1.0	1,400	49	51	..	..	..	6.0	....	....	.....
	1/30/13	800,000	15	1.0	3,500	51	48	1	..	..	4.0	....	....	.....
	2/ 2/13	750,000	20	1.3	2,100	37	62	1	..	..	4.2	....	....	2′ 37″

in the ears, nausea and weakness." Dyspnea was present on exertion, but no cough. For two weeks prior to admission she had been annoyed by a buzzing in the head that seemed to start in the ear and radiate toward the neck. She was very nervous, but had no sweating. The increasing weakness was becoming more and more noticeable.

Examination.—The patient is poorly nourished, acutely ill and looks "nervous." The skin is jaundiced. The eyes show slight exophthalmos, the sclerae have an icteroid tint, but the other eye-signs of Graves' disease are absent. There is a fulness in the thyroid region on palpation. A blowing systolic murmur is present in the gland. The lungs are clear. The heart is normal in outline, regular, forcible and rapid. All the signs of mitral regurgitation are present. Over the precordial area, and especially in the second left interspace, a to-and-fro friction rub is heard. There is edema of the lower extremities, especially on standing. In the right retina there were three fresh hemorrhages. The Wassermann reaction and two blood-cultures are negative.

Course. —The patient had constant fever, between 99 and 102 F. At first the diagnosis was somewhat in doubt; but a careful study of the blood showed that the anemia was of the pernicious type. The reticulated erythrocytes were never higher than 6 per cent. (Chart 6), and with the extreme degree of anemia the prognosis was considered bad. In this case also the resistance of the red blood-cells was increased and the coagulation time was much prolonged. The patient grew gradually weaker and died before a donor for transfusion could be obtained.

A summary of the findings in the six cases is given in the accompanying table.

GENERAL CONSIDERATIONS

It is unfortunately still true that any discussion of the treatment of pernicious anemia must be prefaced by the statement that the disease is an incurable one, and that the instances in which complete recovery is alleged as having followed some particular method of treatment, are to be regarded as explicable either on the ground of faulty diagnosis or insufficient observation. Nor is this altogether astonishing in view of the vagueness of our notions concerning the real nature of the malady. In dealing with other, secondary, forms of anemia, the conditions are different, for we are able to train our therapeutic efforts in two directions, first, toward removing the underlying cause of the trouble, and, second, toward influencing the regenerative functions of the blood-forming tissues. In the case of pernicious anemia, however, our ignorance of its etiology converts all our attempts at causal therapy into the purest empiricism (leaving out of account the mooted cases of bothriocephalus, syphilitic and puerperal pernicious anemia), and impels us to the conclusion that for the present we shall accomplish most by endeavoring to stimulate the flagging hematopoietic energies of the bone-marrow.

Of the many measures having this object in view, transfusion of blood in one form or another seems to offer particularly promising possibilities, and the highly encouraging results reported by many clinicians (as for example, Bovaird,[3] Hürter,[4] Hansen,[5] Weber,[6] Morawitz,[7] Sachs,[8] Gulland and Goodall,[9] etc.) more than justify a wider application of the procedure.

Hürter believes in transfusing early and always recommends the operation just as soon as the blood-picture ceases to show evidence of active regeneration. Undoubtedly, some portions of the bone-marrow retain their functional capacity longer than others, but if the delay is too great these also lapse into a state of inertia from which they cannot be aroused, even by the penetrating stimulus of the hemopoietins in the transferred blood.

The history of transfusion is a long one, and those who are interested in its details will find them set forth in an excellent article on the subject by Werner Schultz,[10] which gives also a complete bibliography up to 1910. That it did not require the era of vascular surgery to popularize the measure, is shown by the fact that in 1875, Landois[11] was able to collect from the literature records of 347 transfusions from one human being to another. At about this period the same great physiologist, together with Ponfick,[12] Panum[13] and others, established the fact that transfusion with animal blood which previously had been widely resorted to, was dangerous, and that clinically only human blood could safely be used. Later the question arose as to whether whole blood or defibrinated blood should be employed, and this has been made the subject of extensive discussion and difference of opinion. Many of the German authors still prefer to inject defibrinated blood, regarding the greater ease of working with it as offsetting the danger of introducing the deleterious substances shown to be present by Köhler,[14] Edelberg,[15] Schultz,[16] and others. Later work by Morawitz,[17] Fuld[18] and Moldovan[19] has proved that these hazards may largely be avoided by allowing the defibrinated blood to stand from one-half to one hour before injecting, for under these conditions the injurious bodies produced during the act of defibrination become altered into harmless modifications. Morawitz[7] and Schultz[10, 16, 20] were also able to show that the greatest danger to be provided against was the presence of untoward bodies of the nature of iso-agglutinins or

isohemolysins in the two bloods, and that, therefore, no transfusion should be done unless the corpuscles and serum of the donor and recipient had first been tested reciprocally. In this connection it is worth noting that Hopkins[21] has reported a case in which, although the cells of the donor were only slightly agglutinated by the recipient's serum, smears made immediately after the transfusion showed extensive destruction of the donor's cells by phagocytes from the recipient. Curtis and David[22] after experimental work on dogs, do not consider defibrinated blood suitable for human transfusion, and Pike, Guthrie, and Stewart[23] have found that on standing a number of hours, defibrinated blood undergoes alterations affecting the corpuscles and impairing its nutritive and bactericidal qualities.

Further evidence of the changes occurring in defibrinated or clotted blood is offered by the work of Stevens and Lee,[24] O'Connor,[25] Brodie,[26] Sollmann,[27] and Stewart and Harvey[28] on the differences in the pressor substances in blood before and after defibrination or clotting. Clinical observation confirms this and various authors, such as Hürter, state that the therapeutic effects of whole blood are distinctly better than those following the use of defibrinated blood.

While the development of the art of vascular surgery to its present high degree, including as it does the possibility of transferring absolutely fresh and, as far as we know, unaltered blood from one individual to another, would appear to make all further consideration of the use of defibrinated blood unnecessary, it must be admitted that from the standpoint of the clinician, these surgical transfusions are still far from ideal. In spite of the various highly ingenious devices that have been suggested for facilitating the operation, it is usually time-consuming, except in the hands of particularly skilful operators, and is a considerable ordeal to both donor and recipient. This fact, together with the sacrifice of a vessel which is entailed, makes donors difficult to find and causes them to hesitate about serving a second time; but the most serious drawback lies in the difficulty of estimating the amount of blood transferred. Counting the recipient's cells and determining the amount of hemoglobin at regular intervals, during the transfusion, should never be omitted, but this is at best only a halting method of gauging the blood-flow.

Both our own observations and those of others lead to the conclusion that more is to be expected from small transfusions repeated at rather frequent intervals, than from a single one of large amount (Weber,[6] Henrot,[29] Hürter[4]). There is even a likelihood that, as in the case of other stimulants, unnecessarily large doses of blood may actually be injurious, and as it were, overwhelm the last flicker of regenerative activity still persisting in the marrow. Or, there is also the possibility that the abrupt change from a state of extreme blood-poverty to one in which there is a great profusion of vigorously functionating cells, by relieving the bone-marrow from the insistent demands to which it has long become habituated, allows it to subside into a condition of hopeless inactivity.

Several possibilities suggest themselves in explanation of the effect of the transfusion. Either the transferred blood is functionally active directly as a transplanted tissue, and its value, therefore, is proportionate to the length of time its elements remain intact, or it is conceivable that it may have an antitoxic action on the etiological factor of the disease. Türk,[30] for example, believes that the new blood, being less resistant than the patient's cells, combines with some putative hemolytic principle present, and so by temporarily protecting the bone-marrow, allows it opportunity to restore some of the lost cells. In the opinion of most authors, however, the transferred blood acts as a stimulant, especially to the blood-forming organs, and at least for a time, revives the power of blood regeneration.

Carnot[31] attempted to increase the effect of these stimulating bodies or hemopoietins by using blood from animals that shortly before had been subjected to a venesection, but while there seems to be some experimental basis for believing that this is possible, further work by Gibelli[32] and by Larrabee[33] has shown that the procedure offers little hope of being valuable clinically. The same thing is true of the efforts of Courmont and André,[34] and other French workers, to obtain increased stimulation of hematopoiesis by administering small doses of hemolytic serums. In this connection it is of interest to note that Walter[35] has reported a case of pernicious anemia treated effectively by repeated injections of blood taken from a patient with polycythemia.

Although, of course, it is perfectly possible and even probable

that the benefit following transfusion is more or less a composite of all these factors, it seems most likely—and this opinion is shared by most writers on the subject—that it is the stimulation of the bone-marrow that is chiefly effective.

While the transferred cells may serve to tide over a more or less acute emergency, their life in the new host is certainly too short to account for the steady rise in red cells that is frequently observed during a considerable period following the transfusion, and it is hardly conceivable that this is merely the result of improved nutritional conditions. The persistence of the new cells has been variously estimated by different writers, but is generally supposed to be about three or four weeks. Our own observations, by means of the method of vital staining, incline us to the view that in most instances they do not last so long, two or three weeks being the usual period in our cases, and during the latter part of this time they are so poor in hemoglobin as to be of comparatively little functional value.

Although some clinicians (Esch,[36] Huber,[37] Mann[38]) have had good results from the subcutaneous or intramuscular injection of defibrinated blood, this has not been our experience, or that of the majority of writers. Both from our own observations and from a survey of the literature, the conclusion seems justified that the optimum effect is to be expected from the repeated intravenous administration of rather small measured amounts of undefibrinated blood. The desideratum appears to be the employment of a method by means of which this can be effected with less difficulty for all concerned than is the case when a vascular anastomosis is undertaken, so that the measure may be made more easily applicable to the conditions of ordinary practice. Over twenty years ago von Ziemssen[39] reported numerous cases in which transfusion was successfully employed by such a method. It consisted in having one operator thrust a needle through the skin into the vein of the recipient, while another operator performed the same action on the donor. Three glass syringes of 25 c.c. capacity were kept constantly in action, one being filled with blood from the donor, the other being emptied into the vein of the recipient, while the third was being washed out with sterile salt solution by a third person before being again applied to the needle in the donor's vein. By this procedure von

Ziemssen was able to transfer any desired amount of unaltered blood and to measure it accurately. Moritz,[40] in 1911, emphasized the advantages of the method and introduced some improvements in the technic which facilitated the puncture of the vein. Still more recently, Lindeman,[41] using the same principle, has devised a set of cannulas by the aid of which even small veins may easily be entered, and the danger of wounding the intima is reduced, and has reported thirty-six transfusions done without a mishap, one of them in a child of seven weeks.

CONCLUSIONS

1. The transfusion of physiologically unaltered blood is one of the most promising forms of palliative treatment available in pernicious anemia. The number of cases on record in which a remission of notable degree and considerable duration has followed immediately on a transfusion is so great as to make it impossible to regard these results merely as coincidences. If proper precautions are taken to select a healthy donor and by the usual tests for isohemolysins and isoagglutinins the serum and corpuscles of donor and recipient are found mutually congenial, there is no danger, and the measure should be employed earlier in the disease instead of waiting until the patient is in a desperate condition.

2. There is evidence in favor of the view that greater judgment and accuracy are needed in determining the amount of blood to be transferred. It is quite possible that too large an amount of transferred blood may be injurious, and that more benefit is to be expected from small doses introduced at intervals to be determined by the progress of the patient.

3. The enumeration of the reticulated cells by means of the method of vital staining affords a useful means of gauging the hemopoietic activity of the bone-marrow, and by watching the patient's progress in this way the indications for and effects of various therapeutic measures can be well determined and supervised.

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THEORIES OF THE ETIOLOGY OF PERNICIOUS ANEMIA

By Karl M. Vogel, M.D.

The etiology of pernicious anemia belongs to that pleasant group of subjects which are always agreeable to discuss because our ignorance concerning them is so extensive that it is very easy to have a great deal to say about them. Indeed, when one considers the enormous amount that has been written relating to this disease and the comparatively small kernel of truth which it contains a certain saying of Goethe's seems particularly appropriate:

Denn eben wo Begriffe fehlen,
Da stellt ein Wort zur rechten Zeit sich ein.

During the past few years, for various reasons, among others the wider resort to splenectomy and transfusion as measures of treatment, interest in the disease has been greatly stimulated, and there is real difficulty in determining which topics to select for discussion. I must, therefore, at once disclaim any intention of attempting completeness in what I have to say in the short space of time that has been allotted, and purpose simply to present briefly some of the views which have lately been developed and which seem especially significant.

Even the most casual allusion to the subject, however, is hardly reasonable without a reference to the changes we are tending to adopt in our conception of the different types of anemias, owing to the somewhat clearer insight we are gaining into their nature, and a comprehension of this altered point of view is necessary in order to understand the relation which pernicious anemia is now believed to bear to the other forms. Even at this date the difficulties in attempting to establish a rational plan of classification are very great, because some of the fundamental facts needed are still lacking, and because we are not altogether certain as to what are the important and basic criteria by which the different groups are to be separated, and what are merely unimportant

and superficial differences. A distinction between so-called primary and secondary anemias has long been current, the former being regarded as true diseases of the blood, and the latter as more or less symptomatic manifestations of other conditions having a well understood nature and etiology. It is clear, however, that this view must be abandoned, for logically considered, there can be no such thing as a primary anemia, since the blood is not itself an organ, but is the direct product of the activity of the various hematopoietic organs or systems, and in a sense may more properly be regarded as a secretion than as an organ. The erythrocytes, having relinquished their nuclei, have no longer the right to be considered as true cells, and even the circulating white cells have only to the slightest degree, if at all, the power of reproduction. So the blood cannot be considered as being the seat of a primary disease any more than can the gastric juice, for example, and it must be conceded that all anemias are, strictly speaking, secondary. To classify them arbitrarily as cryptogenetic or phanerogenetic accordingly as we are acquainted with, or ignorant of, their nature is equally unreasonable, and has not even the merit of expediency, for it leads to such extraordinary associations as placing two of the most antithetical diseases of all, pernicious anemia and chlorosis, in the same group,—surely two of the most uncongenial nosological bedfellows imaginable. The anemic state in the last analysis is the result of a disturbance of the normal equilibrium between blood destruction and blood production, and we find that in one group of anemias it is chiefly the former process which predominates, while in another it is a deficiency of the latter. Therefore a wholly reasonable attitude is to divide the anemias into two broad classes, one of which embraces those types in which the most conspicuous element is a reduction in the production of blood elements, while in the other group the significant feature is their abnormal destruction. To the former belong the anemias due to cachexia, wasting diseases, tumors, and some of the toxic anemias, as well as the very special and distinct form of aplastic anemia, and the interesting type known as osteosclerotic anemia, to which attention has lately been called by Assman,[1] Reich,[2] and others. In the other group come first the anemias due to loss of blood through hemorrhage, either acute or chronic, and the entire class of so-called

hemolytic anemias, such as those caused by hemolytic poisons of definite nature, like pyrodin, phenylhydrazin, nitrobenzol, toluylenediamine, acetanilid, potassium chlorate, etc., and by those of a vaguer character, like the toxins of certain intestinal parasites, malarial organisms, or those sometimes produced in pregnancy, syphilis, and carcinoma. Then there are the various types of hemolytic icterus, and finally the progressive pernicious or Biermer's anemia. Chlorosis, being so essentially a disease *sui generis*, depending most probably on a constitutional anomaly of the internal secretion of the female sexual glands, and differing in such fundamental characteristics from the other forms of anemia can hardly be linked with any of them, and would better be dignified by a separate pigeon-hole all to itself. Pernicious anemia accordingly finds its place with the hemolytic anemias.—that is, those in which the most striking pathogenetic feature is the fact that there is abnormally great cell destruction with a pathologically increased demand on the blood-forming tissues, rather than an inadequacy of these to meet the normal needs. Yet there is more at work than a mere hemolytic agency, for in hemolytic icterus, for example, there are also evidences of severe hemolysis, yet the anemia rarely takes on a very grave character. Evidently, in pernicious anemia the myeloid tissue also is deeply implicated by the hemolytic factor and finally becomes bankrupt under the double strain of supplying the abnormal demands upon its hematopoietic function and in resisting the direct onslaught of the myelotoxic substance. It should be noted, however, that the state of complete marrow exhaustion existing in the so-called aplastic anemia is of totally different nature, and is not to be considered as the end stage of, or an unusually severe form of, pernicious anemia. Aplastic anemia, which is characterized by great reduction in platelet formation and a strong tendency to bleeding is to be classed with the hemorrhagic or purpuric diseases, for it is primarily a leukomyelotoxicosis, rather than a hemolytic anemia, and can be produced experimentally by benzol poisoning or with the *x*-rays. We, therefore, conceive of pernicious anemia as being a chronic hemolytic anemia, the result of the action of certain hemolytic agencies, probably of varying natures, which are distinctive in that they evoke a special type of regenerative response on the part of the blood-producing organs

which gives a perfectly definite clinical picture. Since the blood changes produced and the alterations in the blood-forming tissues both recall the conditions observed in the developing embryo, this so-called reversion to the embryonal type of hematopoiesis is regarded as a fundamental concept in the picture of the disease. These manifestations comprise in the circulating blood the well known factors of high color index, macrocytosis, the presence of megalocytes and megaloblasts, leukopenia, and relative lymphocytosis. At the same time the bone marrow bears evidence to its over-stimulation through the conversion of the normally fatty marrow of the long bones into actively functionating hematopoietic centers with erythroblasts, megaloblasts, and megalocytes, as well as myeloblasts and myelocytes, though the actual output of leukocytes is always reduced. Further evidence of the active efforts at hematopoiesis is offered by the presence, as shown by the important observations of Meyer and Heinke,[3] and others, of extramedullary foci of erythropoietic and myeloid tissue in the spleen, in the liver, and possibly in the lymph glands. As a rule, however, the lymphoid system as such shows no manifestations of stimulation, and the production of lymphoid cells is never increased. It is this simulation of conditions existing in the organs and in the blood-forming mechanism during intrauterine life that constitutes the reversion to the embryonic type of hematopoiesis considered so characteristic of pernicious anemia. On the other hand, in osteosclerotic anemia, in which the bone marrow is gradually crowded out of existence by the encroaching bony new growth, there is also a development of myeloid foci in other tissues, but though the circulating blood shows a high hemoglobin index and megaloblasts may be found, the presence of a leucocytosis and myelocytosis always distinguishes the picture from that of pernicious anemia, since the lesion of the bone marrow is not at all a toxic one.

In dealing with clinical cases it must be borne in mind that the blood picture is subject to great variation during the course of the disease, and that during remissions some of the characteristics, for example, the presence of megaloblasts, may be lacking, so that the other symptoms, such as increased urobilin excretion in stool and urine, yellowish tinge to the skin, fever, gastric symptoms, glossitis, spinal cord lesion, etc., must also be

taken into account in making the diagnosis, while the blood picture alone may also, to a very considerable degree, be simulated in other types of hemolytic or chronic hemorrhagic anemia.

Granted that the disease is a primarily hemolytic one, the question as to the origin and nature of the hemolysins remains to be solved. In some cases the *materia peccans* is definitely known. For example, there is no doubt as to the rôle played in the bothriocephalus anemia by cholesteryl oleate, the cholesterol ester of oleic acid set free by the decomposing segments of this worm, and in the hemolytic anemia of pregnancy a definite hemolytic agent seems to have its origin in the placenta. Numerous authors have described hemolytic extracts obtained from the mucosa of the gastrointestinal tract, spleen, etc., but this evidence is still incomplete, for active blood-dissolving agents can also be obtained from the organs of persons not having suffered from anemia, while the atrophic changes of the gastric and intestinal mucosa formerly considered a typical portion of the picture have been shown by Faber and Bloch[4] often to be absent or very slight if post-mortem changes are avoided by the injection of formalin promptly after death. A curious fact has been pointed out by Hunter,[5] in support of the view that the disease is produced by toxins absorbed from the mouth or gastrointestinal canal and acting through the portal circulation, for it appears that the liver in pernicious anemia may contain many times its normal amount of iron, resulting from red cell destruction, while the spleen shows no such increase, as would be expected if the hemolytic substances acted in the general circulation. That the spleen plays an important role in pernicious anemia, and especially in hemolytic icterus, is also certain, but even the enormous amount of very fruitful work done in this field by Pearce[6] and his collaborators has as yet failed to clear up the situation definitely, and evidently some further extrasplenic factor or rather factors are involved. As Türk[7] puts it, "The hemolytic diseases are the children, and the spleen is their mother, but the father is still unknown, and possibly there are several fathers." Results of some significance have been obtained by Iwao[8] who has been working on amino acids in Sasaki's laboratory in Tokio. By injecting p-oxyphenyl-ethyl-amin into guinea pigs he was able to produce a blood picture closely resembling that of pernicious anemia. That

of itself is not remarkable, since there are other substances that will produce a similar effect, but p-oxy-phenyl-ethyl-amin, or tyramin, which has been isolated from putrifying meat, Swiss cheese, and ergot, can also be produced from tyrosin by the action of various bacteria, notably the colon bacillus, so that we have here the demonstration of the strongly hemolytic activity of a substance which can be, and no doubt is, produced in the human intestine. A recent theory, also implicating the gastrointestinal tract, is that of Cederberg,[9] who believes that pernicious anemia is really a manifestation of anaphylaxis resulting through the absorption from the bowel of body foreign protein, taking as a starting point the observation that animals dying of anaphylactic shock show a certain amount of hemolysis. In pernicious anemia a constitutional defect of the intestinal mucosa is pre-supposed which permits the incompletely broken down albumins to seep through into the blood stream and act as hemolytic agents.

Of interest is the work that has lately been done on the pernicious anemia of horses. This is a disease which is not infrequent in some parts of northeastern France and central Europe. The affected animals develop an anemia which has much in common with the pernicious anemia of human beings. There is an extreme anemia with a high hemoglobin index, intense polychromatophilia, leukopenia, relative lymphocytosis, disappearance of eosinophiles, and reduction of platelets. The bone marrow is hyperplastic and shows evidence of abnormally active hematopoiesis and leukopoiesis. Foci of myeloid cells develop in the spleen and liver, and these organs, as well as the bone marrow, resemble those of embryos before the seventh month. An acute form of the disease is observed which terminates fatally in a week or two, with all the manifestations of an acute infection, while the chronic form may proceed for months or even up to a year. It has been considered that the disease is of an infectious nature, for it can be transmitted to horses and donkeys by the injection of serum from diseased animals, but not to animals of other varieties. The virus is resistant to drying, for the dried serum loses its infectiousness only after seven months, but it is rendered inactive by heating for one hour at 58 degrees C. Observations recently made by Seyderhelm,[10] if substantiated, may prove of some interest in connection with the

etiology of human pernicious anemia. This worker autopsied eighty-one horses suffering from the disease, and found that in every case the stomach contained the larvae of several varieties of fly belonging to the *Oestridae*. It was possible to extract from the bodies of these a toxic agent of great activity, so strong indeed that in one case the injection subcutaneously of an extract of four of the larvae killed a horse in twelve minutes. The behavior of this toxic substance, which Seyderhelm calls oestrin, indicates that it is of purely chemical nature, for it withstands heating in the autoclave. Its administration to horses gives rise to an anemia in all respects resembling the spontaneously occurring disease and the blood of these animals transmits the affection to others, though the newly formed poison differs from the old in being thermolabile. Oestrin therefore appears to be a substance which is able to produce in the body a chemical poison which is capable of reproducing itself when introduced into a fresh animal and Seyderhelm suggests that such toxins, which he terms phyotoxins, may be responsible for the production of some of the other diseases thought to be caused by ultra-visible viruses. In passing, it may be mentioned that Moffit[11] has injected the blood of pernicious anemia patients into horses, but without any effect.

The striking results obtained by splenectomy in pernicious anemia, Banti's disease, and congenital and acquired hemolytic icterus, have directed attention especially to the pathological changes which the organ shows in this group of diseases. These appear to be of a nature to bring the red blood cells more closely into contact with the pulp cells of the spleen,[12] for this portion of the organ is found engorged, while the sinuses are relatively empty. There is a thickening and hyaline degeneration of the follicular arterioles, thus reducing their lumen and apparently side-tracking a considerable portion of the blood stream entering through the central artery, diverting it from the sinuses and causing it to flood the cell columns of the pulp. Here one finds foci of myeloid cells and evidences of phagocytosis, but it is probable that there is not so much actual blood destruction as a sort of sensitization of the red cells rendering them more susceptible to hemolytic action on the way to the liver, or perhaps by the Kupffer cells of this organ. The occurrence of remissions during which

the blood picture suddenly becomes greatly altered for the better proves, however, that these purely organic changes cannot be the sole lesion and that there must be other agencies at work as well. In this connection the studies of Eppinger[12] and King[13] are important, for they found that in conditions associated with hemolysis, such as pernicious anemia and hemolytic icterus, there is an increase in the unsaturated fatty acids of the blood, which are known to have strongly hemolytic properties. Splenectomy, however, has the effect of reducing the amount of these, while the total fats and cholesterol of the blood which are antihemolytic are increased. These observations are highly suggestive, but not altogether in accord with the observations of McPhedran[14] on the relation of unsaturated fatty acids to hemolysis, and further work along these lines is highly desirable. None the less, the effects of splenectomy and transfusion, either separately or combined, are encouraging, and these two measures give the most hopeful outlook in treatment. From the seven splenectomies for pernicious anemia that have been performed at St. Luke's Hospital by Dr. Downes we have gained the impression that the subsequent effects of transfusion were more marked and persisted longer than in cases in which the operation had not been done. Greater care seems necessary in the choice of donors for transfusion, and undoubtedly in time it will be found possible to devise more delicate tests than the relatively coarse ones for hemolysis and agglutination which we now apply. There are surely more subtle factors at work here, concerned perhaps with the colloids of the blood, as suggested by Doerr[15] and Moldovan[16] in connection with anaphylaxis and coagulation phenomena, and as discussed in greater detail in a paper now in press by Satterlee and Hooker from the laboratory of Clinical Pathology at the College of Physicians and Surgeons.[17] Other studies which we have under way in this laboratory will, I hope, aid in determining some of these factors, especially in relation to the effect of transfusion upon the ferment-antiferment balance in the blood plasma of the recipient. That some such considerations must be reckoned with is shown by the fact that sometimes a transfusion from one donor is followed by little or no benefit, while subsequent injections of blood from another individual may result in definite hematopoietic reactions, and it is always advisable, if a trans-

fusion is not attended by good results to select a different donor when the measure is repeated. The benefits of the procedure depend more, I think, on the stimulation that is given to the bone marrow than on the mere pouring into the vessels of a large volume of blood, and I am strongly of the opinion that small transfusions of one hundred to two hundred c.c., frequently repeated, are more likely to be a benefit than massive transfusions given as a last resort when the patient is *in extremis*. In gaging the effects of transfusion and of therapeutic measures in general we have found in our cases that watching the fluctuation of the reticulated cells as brought out by supravital staining is of much interest and definite practical value in measuring the degree of bone marrow reaction. Another very useful guide to the intensity of the hemolytic process and the effects of such measures as splenectomy or transfusion in checking it and also in determining the indications for splenectomy is furnished by the study of the urobilin in the urine and particularly in the stools, or as has lately been suggested by Schneider[18], in the duodenal contents. The steps by which this substance is elaborated from hemoglobin through the intermediate stages of hemochromogen, bilirubin, and urobilinogen, and the utilization of a portion of the urobilin for the resynthesis of hemoglobin through the intermediate formation of a new polymerization product of urobilin called urobilin complex, have been most carefully studied by Addis[19] who has also described a roughly quantitative method for determining the twenty-four hour excretion of urobilin and urobilinogen in urine and stools.

Finally, in addition to confessing that our efforts to cure the disease are still unavailing, we are obliged to admit that, unlike some of the other forms of anemia, notably chlorosis, which are diminishing, it is distinctly increasing in frequency. This has been noted not only in Europe, but also in this country. At St. Luke's Hospital, during the seventeen years from January 1, 1889, to January 1, 1916, we have had one hundred and seventeen cases of pernicious anemia. Of this number twenty-one were admitted within the first period of five years, thirty-two during the second period of five years, and forty during the third period of five years, while during the last two years there have been twenty-four cases, which is at the rate of sixty cases for the coming five

year period. Or, expressed in percentages of the total number of medical admissions, during the first period of five years, 0.33 per cent. of the medical patients suffered from pernicious anemia, during the second period, 0.54 per cent., during the third period, 0.7 per cent., and during the last two years, 0.75 per cent., a rate of increase which certainly is noteworthy.

It is evident that while the etiology and pathogenesis of pernicious anemia are still most perplexing problems, we have gained some knowledge of the separate pieces which must be combined to form the completed puzzle. The disease is a symptom complex based fundamentally on a condition of increased hemolysis plus a toxic action on the bone marrow, but the origin of these manifestations is most involved and depends on a variety of partial causes which are differently associated in different cases. One of these factors appears to be an abnormality of splenic function, conveniently termed hypersplenism, while another is the presence of some hemolytic agent. In occasional instances which we have learned to recognize this agent is a toxin produced by the fish tape worm, or by pregnancy, or by syphilis. In the majority of cases, however, it is still quite unknown, though it is probably of endogenous, rather than exogenous, nature, and there is much evidence in favor of the view that it is derived from the alimentary tract and belongs to the rather indeterminate group of ether-soluble substances termed lipoids. Possibly this lipoid substance serves only to render some other agent actively hemolytic,—as cobra venom, for example, is activated by the lipoid lecithin, with the formation of a hemolytic toxo-lecithid,—or perhaps some action occurs such as has been shown in cases of experimental tolnylenediamin anemia. Here the hemolysis is not caused directly by the chemical itself, but by a newly-formed lipoid produced in the liver as the result of the poisoning and it is interesting to note that if the spleen is first removed much larger doses of the drug are required to produce the effect.[20] In addition there is probably a disturbance of the lipoid metabolism of the body in general, resulting in a decrease of the anti-hemolytic properties of the serum. This may reasonably be associated in our speculations with a disturbance of the functions of the ductless glands, notably the adrenals, for studies on the cholesterol content of the adrenal cortex and

the blood have shown that an intimate relationship exists between the two,[21] while the anti-hemolytic properties of cholesterol have been well established. Still, though it has been found that in pernicious anemia the lipoids of the adrenal cortex are diminished[22] and also that cholesterol protects animals against experimental cobra venom anemia, attempts to use this agent in the treatment of pernicious anemia have been quite fruitless. Furthermore, one is led to believe that there must be some constitutional anomaly, some inherent weakness, either temporary or permanent, which renders the body susceptible and permits the other agencies to take effect. No one of these factors can by itself be termed the cause of the disease, for neither the bothriocephalus, pregnancy, nor syphilis,—oral sepsis, gastric achylia, nor hypersplenism, alone can produce it, and all such entities form only single links of the etiological chain which binds the victim of pernicious anemia to his fate.

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ANALYSIS OF A SIALOLITH OF UNUSUAL SIZE

KARL M. VOGEL, M. D.

The accompanying photograph shows a salivary concretion which is deemed worthy of note on account of its large size and also because complete quantitative analysis of such calculi have very rarely been reported. The patient, J. C., forty-nine years old, of English birth, and a cook by occupation, was admitted to the service of Dr. Lambert on January 28, 1913, suffering from an acute exacerbation of a chronic polyarthritis of five years duration. During the past nine months the symptoms had been so severe as to compel him to stop work on several occasions, the ankles and shoulders being especially involved. Under treatment, which comprised oral hygiene, baking, and the administration of alkalis, aspirin, and atophan, his condition rapidly improved, and on February 20th he was discharged free from any symptoms.

Apart from the condition of the affected articulations the physical examination showed little that was noteworthy, except the very neglected state of the mouth. The teeth were poor and the gums also in very bad condition. Attached to the alveolar border of the upper jaw was the concretion in question. This was loosened and removed without great difficulty by Dr. Patterson. Before it was sawed across to expose the interior and to obtain material for analysis it weighed 4.2 gm. and measured 2 x 1 x 2.75 cm. On analysis its composition was found to be as follows:

CaO	41.5%
MgO	5.7%
P_2O_5	39.3%
CO_2	1.9%
Material insoluble in HCl and HNO_3	6.4%
Water	4.3%

Sialolith

DETECTION OF MERCURY IN THE EXCRETIONS.*

KARL M. VOGEL, M.D., and O. I. LEE, B.S.

The identification of mercury in the urine, stomach-contents, or feces of persons supposed to be suffering from poisoning by this metal is often a matter of great diagnostic importance, and is of especial significance owing to the fact that if the proper eliminative treatment is begun without delay the patients' lives can be saved in a considerable proportion of the cases. The newspaper notoriety given to recent cases of poisoning with mercuric chlorid, the ease with which the substance may be obtained, and the mistaken but prevalent idea that the death caused is rapid and without discomfort, have procured for this agent a wide vogue among those attempting suicide; while its popularity as a household antiseptic causes accidental poisonings to occur with considerable frequency. The history to be obtained from such patients or their friends, however, is often extremely unsatisfactory, and there is nearly always room for doubt whether or not the poison has actually been taken, and if so, whether the amount is large or small. Most of the cases of accidental poisoning occur at night through the victim's having swallowed something from a bottle in the dark, and often there is merely the suspicion that what was ingested came out of the poison container and not from its innocent neighbor, so that the patient and his friends remain in a state of mind which is uncomfortable, to say the least. Would-be suicides, on the other hand, frequently deny the nature of the substance taken, and the only proof possible may be that of demonstrating the poison in the stomach-washings or in the urine or stools. As a result the occasions in which it is necessary to test for the presence of mercury in the excretions are not so very rare, but unfortunately most of the methods available for this purpose

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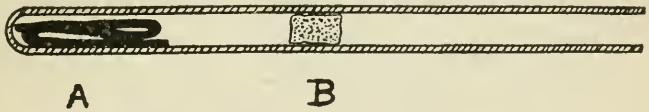
are far too complicated to be suitable for clinical application. In two cases of mercuric chlorid poisoning, one with suicidal intent, the other accidental, recently treated at St. Luke's Hospital, and in a third from outside, the following procedure, which is a combination of various steps from several older methods, was found to be very satisfactory in identifying the poison and following its elimination in stomach-contents, urine and feces.

In dealing with urine, about 150 c.c. is taken, and in order to break down the organic compound in which the mercury is likely to be present, it is acidulated with 5 c.c. of concentrated hydrochloric acid and evaporated over a free flame until its bulk has been reduced to 25 or 30 c.c. About 2 c.c. of hydrochloric acid is added to replace the loss by evaporation, and enough potassium chlorate to oxidize thoroughly the organic material present. This usually requires about 2 gm., and when it has been effected the fluid becomes pale yellow or colorless. It is then diluted to about 60 c.c. and is boiled vigorously until the chlorin gas previously evolved has been driven off, which is shown by the absence of chlorin odor from the steam. The solution usually darkens again on cooling. A piece of copper wire (about 18 gage) about 4 cm. in length, bent back on itself twice and cleansed by boiling a short time in a test-tube with dilute hydrochloric acid, is dropped into the solution and allowed to remain for an hour or more. If considerable amounts of mercury are present it will then be found to be coated with a silvery film of metallic mercury; but this is not sufficient to establish the identity of the metal, and if it exists only in traces the changes in the appearance of the wire may be inconclusive. The wire is accordingly removed from the dish with a glass rod, is washed with a little water and is gently dried by rolling it on a piece of filter-paper, pains being taken to avoid unnecessary handling and attrition. It is then allowed to slip to the bottom of a glass tube from 3 to 5 mm. in diameter and 15 cm. in length, which is sealed at one end, and is followed by a cylindrical plug of gold-leaf which is pushed into the tube until it is within 2 cm. of the wire. Such pellets of gold-leaf are used in filling teeth and are furnished by the dental supply-houses in the size required for the present purpose, so that when needed it is easy to obtain them from the nearest friendly

dentist. The arrangement of the objects within the tube is shown in the accompanying sketch.

Holding the tube horizontally, the end containing the wire is gradually heated by brief successive introductions into the small flame of a Bunsen burner or spirit lamp, care being taken to avoid heating the part of the tube containing the gold-leaf. The latter must be examined frequently for any change of color, especially the end of the cylinder toward the wire. If mercury is present it will manifest itself by the appearance of a silvery patch of amalgam in this situation. If the amount of the metal



Apparatus for the detection of mercury in the excretions; A, copper wire; B, pellet of gold-foil.

is exceedingly minute there will be simply a pale discoloration of the gold, seen to better advantage with the hand-lens or by removing the pellet from the tube and examining it with the low power of the microscope; but if the amount is larger the deposit on the gold will be very easy to recognize.

If the pellet is too close to the wire the heat traveling along the tube may cause a deposit of mercury which at first was distinctly visible to become diffused so that it grows less evident. In doubtful cases the experiment should be repeated, using larger quantities of the material to be tested and allowing the wire to remain longer in the solution. If a positive result is obtained the tube may be sealed and kept as a permanent record. If further confirmation of the identity of the mercury is required, the gold-foil may be suspended in a tube containing a few crystals of iodin which are then very gently warmed. The mercury thus becomes converted into red mercuric iodid; or if the amount is considerable the metal may be distilled by heating the gold-foil in the tube and looking with the lens for a deposit of very minute droplets of metallic mercury in the cooler parts of the tube. Some idea of the amount of mercury present may be gained by weighing the gold before and after the formation of the amalgam.

In testing stomach-contents or feces the same method is employed although the quantity of material taken is usually

smaller. The material must be well mixed to insure getting a uniform sample of the specimen, especially in the case of stomach-contents if egg albumin has been given as an antidote, as the mercury is then in the form of albuminate. The oxidation also takes longer, and larger quantities of potassium chlorate are required. Before the wire is placed in the solution, the latter should be filtered in order to remove any fatty substances, carbon, or other insoluble materials.

Corrosive sublimate is readily reduced by organic matter to metallic mercury, and the latter, as well as mercuric chlorid, is volatile to a slight extent with steam, so that this must be kept in mind in evaporating or digesting solutions which may contain very minute amounts of the poison. The quantity of mercury which the copper is capable of abstracting from the fluid is a function not only of the concentration of the solution but also of the time of contact, so that if the test is negative after the wire has been immersed for one or two hours, another piece of wire should be dropped into the fluid and allowed to remain for a longer time, even over night.

In order to test the sensitiveness of the method the following experiments were done: A solution of mercuric chlorid was made containing 0.0001 gm. of mercury to the cubic centimeter, equaling 0.01 per cent. or 1:10,000. Using this solution as a basis, successive dilutions showed that a definite positive reaction could be obtained when a solution containing 0.00002 gm. of mercury in the dilution of 1:1,000,000 was used, allowing the wire to remain in the solution three hours.

In order to make the controls more nearly resemble clinical conditions, experiments were made using three different specimens of albuminous urine, and two specimens each of stomach-contents and stools, to which definite amounts of the mercury solution were added, the mixture being allowed to stand over night before the tests were made. It was found that 1 mg. of mercury in 100 c.c., equaling a dilution of 1:100,000, could be detected in the urine, stomach-contents, and stools, the wire being allowed to remain in the fluid two hours. In these cases the amount of amalgam formed on the gold was sufficiently large to be recognized with the unaided eye. Further control tests with materials to which no mercury was added gave negative results.

The urine of twelve patients who had each received 2 grains of calomel the night before was also tested. In two of these a very faint positive reaction was obtained, showing the delicacy of the method, and also, that it is desirable before making a diagnosis of mercury poisoning to find out whether or not the patient has been given calomel.

The principle of securing disposition of the mercury on a metallic surface is made use of in many of the standard tests, such as the classical one of Reinsch and those described by Jolles,[1] Winternitz,[2] Almen,[3] Brugnatelli,[4] Mergte,[5] Mauthner,[6] and Ludwig and Zillner.[7] All of these, however, involve complex manipulations or the use of reagents not always available, such as gilded platinum, powdered reduced copper, gold chlorid, etc. The formation of gold amalgam as a means of recognizing minute amounts of mercury also is not new, but it is believed that the technique suggested in the foregoing offers a simple, fairly rapid and exceedingly delicate method of testing for mercury which can easily be carried out by any physician.

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CHORIOEPITHELIOMA OF THE TESTIS

WM. H. WOGLOM, M. D.

Before entering upon a description of chorioepithelioma of the testis, it will be advantageous to review what is known of uterine chorioepithelioma and of teratoma.

A chorioepithelioma, as its name implies, is a tumor originating in the chorion*, a villous membrane surrounding the entire young ovum and consisting of an outer ectoderm (trophoderm) with an inner mesoderm. At a later stage in the development of the ovum, the villi disappear from all the chorion except that portion which in man becomes attached to the uterine wall.

The chorionic villi first appear as simple projections from the outer layer of the chorion, and are composed entirely of epithelium; before long, however, they branch out into a tree-like form while, at the same time, mesoderm grows into them to form a supporting core. Their epithelium, made up at first of clearly demarcated cells, soon differentiates into two layers which together are known as the diplotrophoblast—an inner, composed of definite cells which divide by mitosis (cytotrophoderm, or layer of Langhans) and an outer, consisting of a rim of protoplasm containing a row of nuclei which appear to divide by amitosis, since karyokinetic figures are not discoverable in them. In this outer layer, the syncytium or plasmoditrophoderm (more properly, perhaps, syncytiotrophoblast), there are no cell boundaries. Being amphophilic, it assumes a purplish hue in sections stained with eosin and hematoxylin, while the cytoplasm of the Langhans' cells has a foam-like appearance, conferred by the dissolving out of their glycogen globules during fixation. The syncytium, which is about equal in thickness to the cell layer of Langhans, is provided on its outer surface with a delicate membrane composed of stiff hair-like processes, the stereocilia or

* The following description of the chorion is from the textbooks of Bailey and Miller and Keibel and Mall.

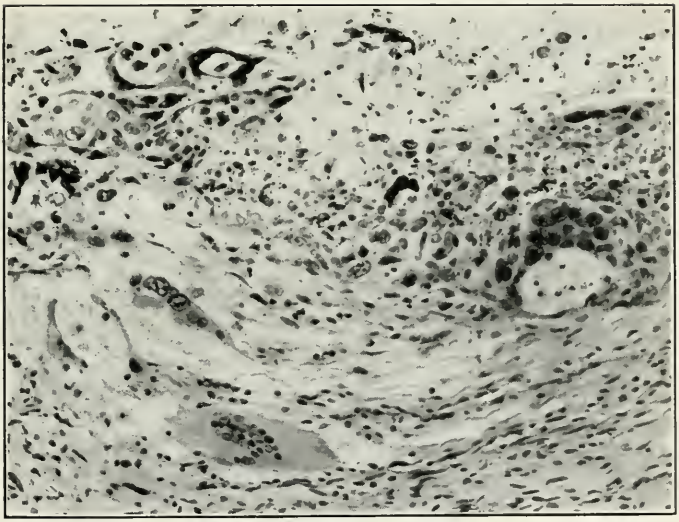

Fig. 1. Case 68. Syncytium. x150

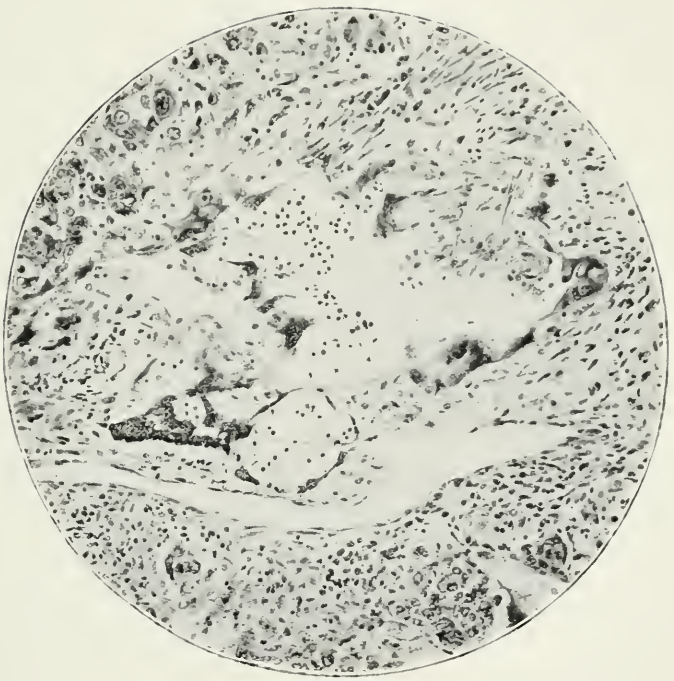

Fig. 2. Case 68. Syncytium and Langhans' cells in intimate relation with blood vessels. x 140

Bürstensaum (Fig. 12), which, however, cannot always be seen.

The trophoblastic origin of the syncytium is upheld by all recent investigators. An older view derived it from maternal endothelium, but such gradual transitions between the cytotrophoblast and the syncytium as the passage of nuclei from the former into the latter have since then been described, while no indubitable evidence of similar transitions between the syncytium and the endothelium have been discovered, a fact of which the importance will become apparent later in the paper. The fetal origin of the syncytium is accordingly no longer in doubt.

It is the province of the trophoblast to invade and open up the vascular channels of the uterus by the lytic power of its cells, and thus to obtain nourishment from the maternal blood for the growing embryo; and this invasive power, retained and increased when the cells pass over into chorioepithelioma, explains the hemorrhagic character of these tumors and their extensive metastasis by way of the blood stream.

Chorioepitheliomata follow normal pregnancy in a few instances, but more often they occur after abortion, and, most commonly of all, in connection with hydatiform mole.

Marchand[1] distinguished two varieties of chorioepithelioma—the typical and the atypical. In the former, the characteristic appearance of the chorion in early pregnancy is not altered: the tumor is made up of the clear, polyhedral Langhans' cells surrounded by bands of syncytium. In the atypical chorioepithelioma, on the other hand, the characteristic arrangement of the two layers is not preserved, and the tumor is composed of deeply staining isolated cells which are polymorphous to the highest degree, and often contain enormous nuclei and resemble giant cells; a few of them may fuse, but large syncytial masses are not often formed. Among these elements, cells of the Langhans type may be found. A transitional variety between the two just described contains occasional large clumps of syncytium.

The next step in our recapitulation must be a review of the teratomata.

After fertilization, the ovum divides into two daughter cells, which soon undergo mitotic division, producing four cells; the

four again divide to produce eight, and so on. These cells are the blastomeres, and in this early stage of their life history they are said to be totipotent, since any one of them can give rise to descendants able to evolve all the tissues characteristic of the adult organism; they are in this respect different from their descendants, which become gradually multipotent, and finally unipotent, or capable of building one tissue only.

In the opinion of Marchand and Bonnet, embryomata of the ovary and testis originate in unused blastomeres at a very early period in the development of these elements, the period of cleavage, a view with which Wilms wholly agrees; hence these growths contain derivatives of all three germ layers, and approach more nearly than any other to the normal architecture of the adult organism. Elsewhere in the body, also, there occur neoplasms containing products of all three layers, regarding which Wilms partially subscribes to Marchand's belief that they are analogous with the embryomata of the testis and ovary just mentioned.

A subdivision of the embryoma group comprises the mixed tumors, simpler than those of the preceding class, though still very complex in their structure. Under this heading Wilms includes mixed neoplasms of the kidney, cervix, vagina, bladder, vas deferens, palate, and the parotid, submaxillary, and mammary glands. These also have their inception in unused blastomeres, differing from the embryomata only in developing at a somewhat later stage in the life of these elements—that of the three germinal layers.

Although Wilms, in accounting for the origin of the embryomata, accepts in part Cohnheim's hypothesis of tumor genesis, he does not agree that the misplacement of embryonal remnants is the sole etiological factor; if it were, he argues, an accessory spleen would develop into a tumor.

Not all authorities, however, agree with Wilms in ascribing these neoplasms to misplaced blastomeres. Among the dissentients is Adami, who believes that one of the strongest arguments adduced by Wilms, namely, the discovery of five separate embryomata in one ovary, is, in fact, a weak point in the hypothesis, since it suggests a dislocation and transportation of the none too numerous blastomers into one particular locality, altogether too

Fig. 3. Case 68. Syncytium partially lining a blood-space. x 115

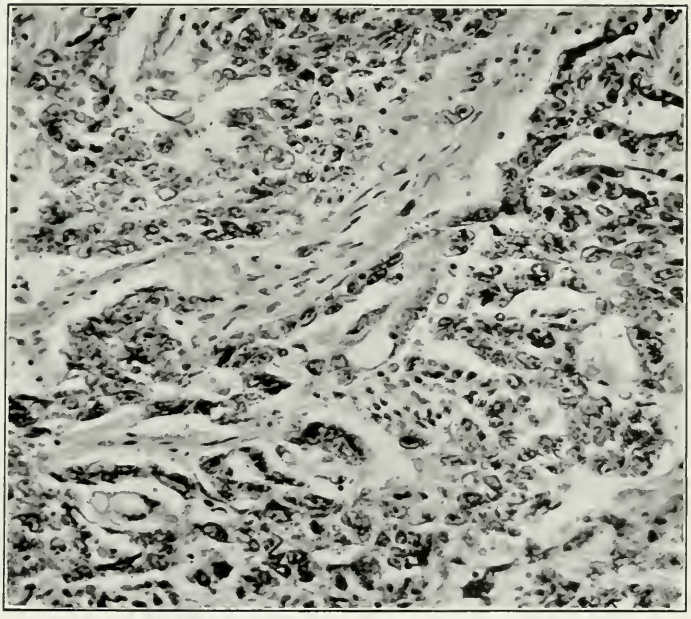

Fig. 4. Case 68. Area of carcinoma. x 200

extensive to be within the bounds of probability. He himself accepts the hypothesis that the embryoid tumors have their origin in a special system of blastomeres, those, namely, of the germinal group. These are dedicated from the first to reproduction—that is, to the elaboration of cells which shall reproduce all the tissues of the parent; and since, furthermore, the total number of germinal blastomeres in the segmenting ovum does not find its way to the ovary or the testis, some becoming dislocated in the process of development, as Beard and others have shown, these cells fulfill all the needs of the case. Thus these germinal blastomeres, as distinct from Bonnet's primitive blastomeres of any order, afford, in his opinion, an adequate explanation for the evolution of three-layer teratomata in the abdominal and thoracic cavities and in still other regions, teratomata of the same type as those affecting the ovary and the testis. In the ovary and the testis, however, he does not consider it necessary to postulate that embryomata arise from germinal blastomeres which have lain unchanged from their very inception; on the contrary, it is probable that any cell along the direct line between the primitive germinal blastomere and the mature ovum or spermatozoön (these last being excepted) may potentially give origin to an embryoma.

Leo Loeb[2] would refer the teratoid and chorioepitheliomatous neoplasms of the ovary to parthenogenetically developed ova, rather than to misplaced blastomeres.

But whatever their origin, teratomata contain representatives of the three germ layers, at least in theory. Actually, however, derivatives of all three are not invariably present; the tumor may contain products of but two of them, or of one only, either because those not represented have never developed, or, having developed, have been subsequently overgrown and repressed by the others.

Since all three layers are potentially present, the teratomata, like the normal embryo, must tend to develop a chorion, and this tendency explains, to the satisfaction of almost all authorities, the presence of chorioepitheliomatous tissue in teratomata. Sternberg[3], however, does not accept this hypothesis, and refers this tissue to the blood-vessels (Fig. 3), for the following reasons. Syncytium-like tissue has been found in other tumors (carcinoma, endothelioma) by several observers, and Sternberg himself

has seen syncytium developing from the maternal blood-vessels in early pregnancy. In the latter instance, his experience is at variance with that of the majority of investigators, who deny any such connection, as has been pointed out in a preceding paragraph.

It is necessary to distinguish sharply, wherever possible, between true chorion and chorion-like tissue. A striking resemblance to chorioepithelioma may be conferred upon carcinoma, for example, by extreme rapidity of growth, a fact to which attention has been called by Risel[4], Mori[5], and others; hence every tumor reported as a chorioepithelioma may not have been, in fact, such a growth. But that the diagnosis was justified in most instances is suggested by the presence of stereocilia or other characteristic features.

Chorioepithelioma of the testis was first observed by Malassez and Monod in 1878, but they called it *sarcome angioplastique,* attributing vasoformative properties to the large syncytical complexes. Although a few later observers, like Sternberg, hold a similar view, the majority accept Schlagenhaufer's hypothesis[6], that the chorioepitheliomatous material is derived from fetal membranes or remnants of these. The question has been asked, and perhaps with some justice, why villi are not discoverable in chorioepitheliomata of the testis, if these tumors actually do originate in this way. An answer may be found in the cases reported by Launois, Masson, and Pinard[7], and by Fritze[8], who found, in metastases, structures so closely resembling chorionic villi that they were often quite indistinguishable from those normally belonging to the fetal membranes. Unfortunately the former case is not entirely conclusive, because the authors were unable to examine the testis, for some reason not stated. They had no doubt, however, that it was the seat of a chorioepithelioma, as it had been enlarging gradually for a year.

Testicular chorioepitheliomata are described by Risel (l. c.) as follows. Both the primary growth and its metastases are hemorrhagic, resembling a mixture of old and fresh blood clots, and are thus very similar in appearance to the chorioepithelioma of pregnancy. As in these latter tumors, the hemorrhage is caused by invasion of the vessels, and metastasis takes place by way of the blood stream.

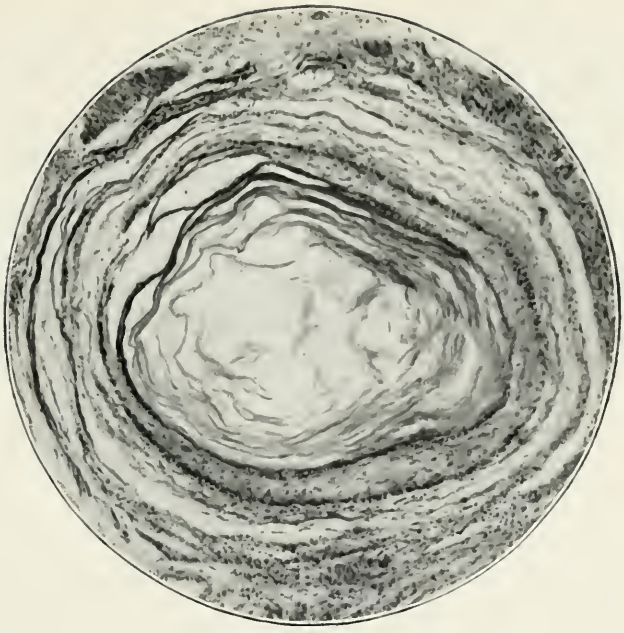

Fig. 5. Case 69. Squamous epithelium and keratin, representing the ectodermal elements. x 75

Fig. 6. Case 69. Glandular epithelium elaborating a mucoid secretion (Gastrointestinal tract?) and representing an entodermal element. x 400

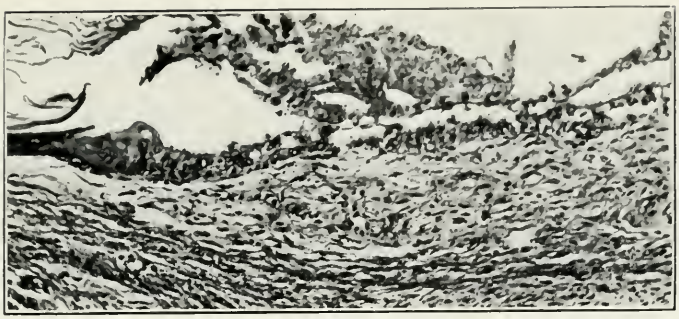

Fig. 7. Case 69. Transition between squamous and glandular epithelium, a characteristic feature of teratomata. x 145

Fig. 8. Case 69. Transition between squamous and glandular epithelium. x 75

The teratoid nature of chorioepitheliomata of the testis is not always easily demonstrable, for the chorioepitheliomatous portion may almost or quite overgrow and repress the teratomatous constituents; on the other hand, a small area of chorioepithelioma in a teratoma may be readily overlooked.

Since the appearance of Schlagenhaufer's paper, a number of other cases have been recorded. Frank[9], who carefully reviewed the literature in 1906, was able to find nineteen, to which he added three of his own; another survey was made in 1908 by Cooke,[11] Ohkubo,[10] while writing in 1915, recorded forty-six cases, and added the forty-seventh. He recapitulated the clinical characteristics of these cases as follows: The age of the patients varied from 16 to 46 years, though the great majority of the tumors occurred in individuals between 20 and 40 years of age. In two instances, the growth was found in an undescended testis. The duration of the disease was very variable, but was generally less than a year; the longest period was 2½ years, and the shortest two months. The prognosis is always grave; in 29 cases a fatal termination was specifically mentioned, one patient appeared to be well five months after operation, while in the remaining 17 the result was not given. The local symptoms are the presence of a tumor that often causes a dull, dragging pain; the general, which are often the first to excite the patient's apprehension, depend upon the localization of the metastatic growths. By far the most common are hemoptysis and dyspnea, while not infrequently the abdominal lymph-nodes may be so extensively enlarged by metastases as to cause intestinal obstruction; secondary growths in the lungs, stomach, or intestine may give rise to fatal hemorrhage. A sign that has been described so far only by Cooke and by Warthin[12] is enlargement of the breasts, a condition which surgeons will undoubtedly seek with great interest in the future. In the case reported by the latter author, an attempt at the formation of acini with the presence of fat in the cells was observed, together with an active growth of connective tissue.

The accompanying table includes 69 cases, all, it is believed, that have so far been reported. It does not in any way alter Cooke's summary of the main facts, except that it adds one further instance in an undescended testicle. The cases of Stokes[13] have not been counted, for it was felt that they were hardly definite

enough for inclusion*. On the other hand, it will be observed that three (in addition to the two cases in Cooke's table) have been added, in which the testis was not examined; in all these, however, the authors stated definitely their belief that the primary growth had been in the testis. In case 53, the testis had been enlarging for a year; in 46, it had been removed for what was diagnosed histologically as carcinoma, though Ohkubo had reason to believe that the growth was actually an embryoma. Only in case 59 is there a really serious doubt. This was an intracranial chorioepithelioma with extensive involvement of the lungs, liver, kidneys, and retroperitoneal lymph-nodes, previously reported in brief by Boestrom[14] and not generally admitted to be of testicular origin. The first symptoms were those of a brain tumor, and Pick[15] suggested that the growth may have been a chorioepitheliomatous teratoma primary in the brain. To this hypothesis, however, Askanazy objected on the ground that cerebral neoplasms rarely give rise to metastases at a distance. Hahn,[16] who has reëxamined the question with great care, furthermore, points out that even though clinical examination repeatedly failed to discover any testicular enlargement, the possibility of a small tumor having been present is not eliminated. On the contrary, he believes that the primary growth actually was in one of the testes, his main reason being that the retroperitoneal nodes which were invaded, are the very group most apt to be attacked by malignant testicular neoplasms. On the basis of Hahn's very thorough investigation, it seems proper to include the case in the table, tentatively at least.

The cases to be reported in the present paper are two in number (68 and 69 in the table). For the former, no clinical data are available. The latter was a man thirty-five years of age, whose right testicle, according to his account, had always been larger than the left. During his boyhood, it had been injured in a game of football, and about ten months ago was struck again; since this injury, he has noticed a continuous increase in its size, accompanied at first by a dragging sensation only, but now by pain. Infection with syphilis occurred fourteen years

* Through the courtesy of Dr. Stokes, it was possible to examine the slides from these two cases, and I wish to acknowledge here my indebtedness to him.

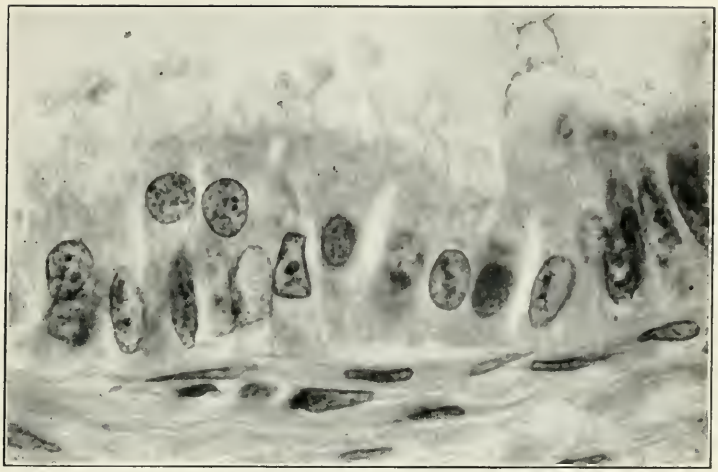

Fig. 9. Case 69. Ciliated epithelium. · Respiratory tract? x 1000

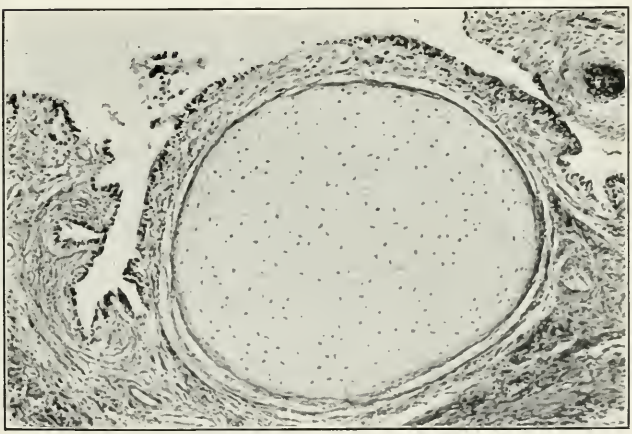

Fig. 10. Case 69. Cartilage and, just above it, a structure possibly representing synovial membrane. x 75

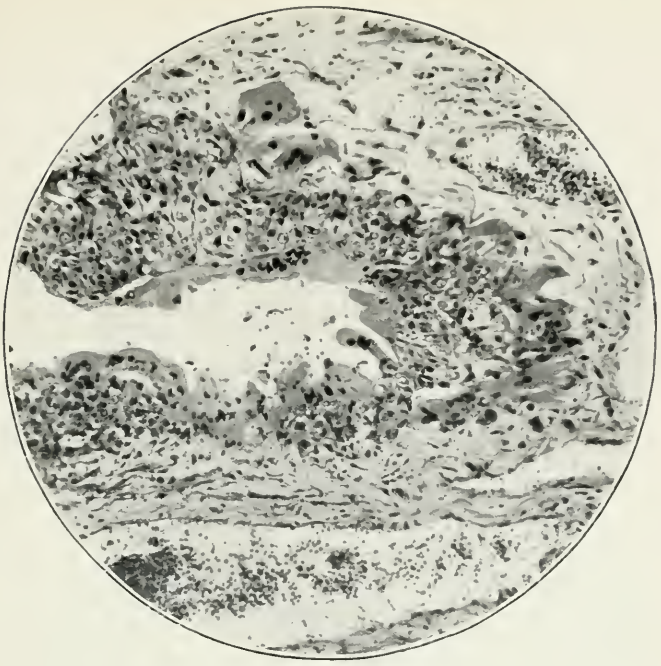

Fig. 11. Case 69. Syncytium. x 145

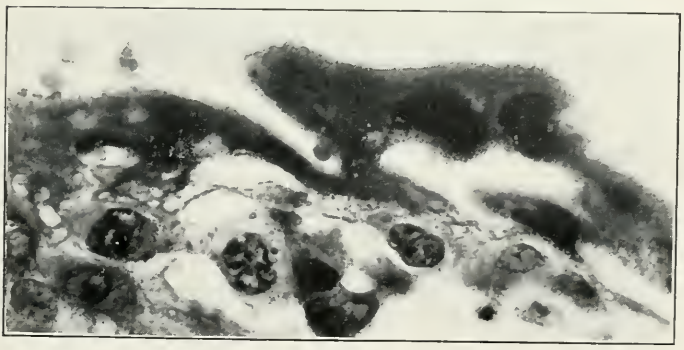

Fig. 12. Case 69. Stereocilia (Bürstenaum). x 600

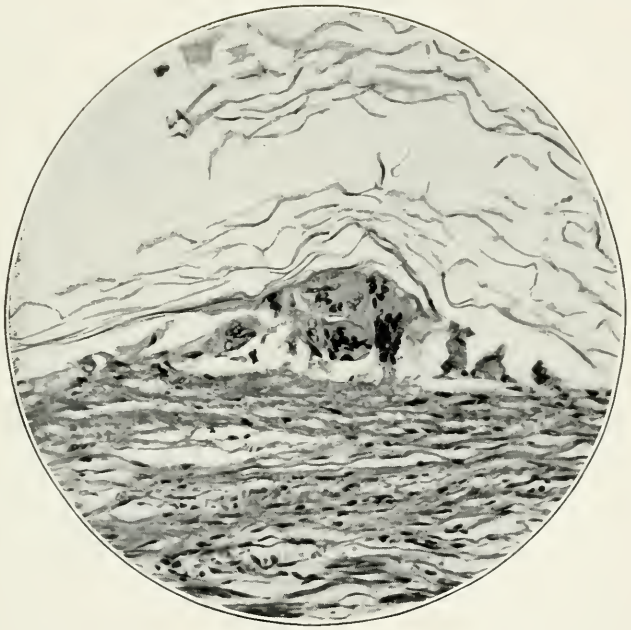

Fig. 13. Case 69. Foreign-body giant cells. x 200

No.	REPORTER	AGE	SIDE	DURATION	RESUL
1	Mallassez and Monod	27	Left	1 yr.	Deat
2	Carnot and Marie	37	"	10 mos.	"
3	Dopter	28	Right	6 mos.	"
4	Wlassow	?	?	?	?
5	"	?	?	?	?
6	"	?	?	?	?
7	"	?	?	?	?
8	Schlagenhaufer	43	Left	?	Deat
9	Breuss and Schlagenhaufer	40	Right	2½ mos.	"
10	Carey	?	?	?	?
11	Schmorl and Steinert	22	Left	?	Deat
12	Schmorl	17	?	?	?
13	Steinhaus	32	Left	6 mos. —	?
14	Risel	20	"	?	?
15	"	35	?	?	Deat
16	Emanuel	26	Left	4 mos.	"
17	Westenhoeffer	30	?	?	"
18	Chevassu	30	Left	3 mos.	?
19	A. Hansemann	28	Right	?	?
20	Askanazy	24	Right	1 yr.	Deat
21	Sternberg	26	Right	1 yr. 2 mos.	"
22	Scott and Longcope	45	"	2 mos. †	"
23	Dillman	32	Left	4 mos. †	"
24	Frank, R. T.	40	?	2 yrs.	?
25	"	16	Left	2 mos. †	?
26	Spilsbury	28	?	8 wks.	?
27	Hicks	30	Right	1 yr.	?
28	Lawrence	?	?	?	?
29	Reinhold	30	Right	7 mos.	Well
30	Bernstein	34	Left	4 mos.	Deat
31	"	29	Right	?	"
32	Bonney	?	?	?	?
33	"	?	?	?	?
34	Orton	38	Left	5 mos.	Deat
35	Monckeberg	19	"	10 mos.	"
36	"	34	Right	?	?
37	"	Young	?	?	Deat
38	Nicholson	30	Right	?	"
39	"	20	Left	9 mos.	Recurre
40	"	25	"	2 yrs. ?	Deat
41	Chuvin	20	Right	6 mos.	"
42	Jeanbrau and Massabuau	44	Right	1 yr.	?
43	Fischer	37	"	?	Deat
44	Ohkubo	26	Right	1 yr.	?
45	"	32	"	?	?
46	"	45	"	10 mos.	Deat
47	Taylor	28	Left	8½ mos.	"
48	Fink	35	?	?	"
49	Klippel and Monier-Vinard	?	?	?	"
50	Gruner	25	Left	5 mos.	?
51	Glaserfield	23	"	2½ yrs.	Deat
52	Sigl	34	Right ?	6½ mos.	"
53	Launois, Masson, and Pinard	40	Left	About 1 yr.	"
54	Kaufmann	43	?	?	"
55	Conforti	46	Left	2¼ yrs.	"
56	Zenoni	32	Right	?	"
57	Marcora	29	Left	1 yr.	"
58	Keenan	20	Right	7½ mos.	"
59	Hahn (Boestrom)	40	?	?	"
60	Tirumurti	35	Right	2 mos.	"
61	Frank, A.	30	Left	2 yrs.	?
62	Meyer, Rudolf.	23	Left	14 mos.	Deat
63	Roncali	18	"	10 mos.	"
64	"	Young	Right	?	"
65	Warthin	35	"	?	"
66	Fritze	21	Left	2 mos.	"
67	Cooke	26	Right	?	"
68	Woglom	?	?	?	?
69	"	35	Right	19 mos.	Deat

OTHER TERATOMATOUS GROWTHS IN		
TUMOR	METASTASES	REMARKS
None	None	Other teratomatous elements not looked for.
"	"	" " " " " "
"	"	" " " " " "
"	"	No clinical data.
"	?	" " "
Present	?	" " "
"	?	" " "
"	None	
"	"	Interpreted as hydatidiform mole.
"	"	No clinical data.
"	Present	
"	?	
"	?	
?	?	
?	Present	Original tumor not examined.
Present	?	Atypical forms present.
"	Present	
None	?	
?	Present	Original tumor not examined.
None	?	Atypical forms present.
"	None	
"	"	Tumor of undescended testicle.
Present	"	
None	?	
Present	?	Atypical forms present.
"	?	Not seen after operation.
"	?	Well three months after operation.
"	?	
"	?	Well five months after operation.
None	None	
"	"	
"	?	
Present	None	
None	"	
"	"	
"	?	
Present	None	
?	?	Slide contains unstriped muscle also, but no statement is made whether specimen is from primary or secondary tumor.
Present	?	Recurred in two months. No further history.
"	?	No statement regarding teratomatous growths in metastases.
None	None	
Present	?	Well about a year after operation.
"	Present	Tumor of undescended testicle.
Present	?	No clinical data after operation.
"	?	" " " " "
?	Present	Primary tumor not examined.
Present	None	
None	?	
Present	Present	No clinical data.
"	?	
"	None	
None	"	Several congenital abnormalities.
?	"	Testicular growth not examined.
None	"	
"	"	
"	"	
Present	?	
None	None	Tumor involved only epididymis.
?	"	Previously reported briefly by Boestrom. Testis not examined.
None	"	Tumor of undescended testicle.
Present	?	
Present	None	Primary tumor contained no chorioepithelioma.
"	?	Connective tissue from mesenchyme in primary. No statement regarding teratomatous material in metastases.
"	?	No statement regarding teratomatous material in metastases.
"	None	Hyperplasia of breast.
Present	Present	Most of the metastases pure chorioepithelioma, though primary contained no chorion.
None	None	Hyperplasia of breast.
Present	?	No clinical data.
"	None	

ago, at which time he submitted to but seven or eight weeks' treatment. Since the swelling was noticed, ten months ago, he has had salvarsan and mercury.

Physical examination disclosed a large and rather firm mass which seemed to correspond with the right testis. This was removed by operation in September, 1915. The man died nine months later with all the signs of pulmonary involvement.

After fixation in formalin, the tumor appeared as an oval mass 9 x 7 x 9 cm. and covered by a smooth layer of the tunica albuginea; the cord was still attached to its pole. The capsule was not over 1 mm. in thickness, and in some places was much less. There had been some hemorrhage into the sac. On cross section, about one-third of the cut surface was found deeply discolored by blood in consequence of hemorrhage into a softened area at the center of the growth. The remainder of the tumor was composed of firm, whitish tissue containing numerous cysts, which measured from about 3 mm. in diameter to almost microscopic dimensions. There remained no evidence of any testicular structure in the gross specimen.

Neither of these two cases requires extensive histological description, since neither adds anything to what is already known regarding the type of growth now under discussion.

The tumor in case 68 contained cartilage, squamous and glandular epithelium, and a small amount of syncytium, the last named shown in Figs. 1, 2, and 3. In the lower left-hand portion of Fig. 3 a structure is to be seen which might suggest the development of syncytium from endothelium, but which is more readily interpreted as a blood-space within an area of syncytium.

This growth contained also an area of carcinoma (Fig. 4), as teratomata of the testis not infrequently do.

In Case 69 there were found squamous epithelium (Fig. 5), glandular epithelium (Fig. 6), transitions between the two (Figs. 7 and 8), ciliated epithelium (Fig. 9), cartilage (Fig. 10), and a much larger amount of syncytium (Fig. 11) than the other tumor contained. Fig. 12 shows very clearly the stereocilia. A group of foreign-body giant cells which might, perhaps, be mistaken for syncytium, is shown (Fig. 13) at the edge of a cyst lined by keratin.

The nervous system did not appear to be represented in either growth.

Both tumors contained, therefore, derivatives of the mesoderm (cartilage) and the ectoderm (squamous epithelium). The glandular and ciliated epithelium may have been either ectodermal or entodermal in origin.

For a full bibliography, the reader is referred to the reviews mentioned in the text.

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A METHOD FOR THE DETERMINATION OF THE TOTAL CHLORINE IN BLOOD

O. I. LEE, B. S.

Solutions:

1. Silver Nitrate.
   The silver nitrate solution is made of such a strength that 1 c.c. is equal to 1 mg. of chlorine. For this purpose 4.7943 grams of pure silver nitrate are weighed out and dissolved in a liter of distilled water.
2. Indicator.
   The potassium chromate indicator is made by dissolving 5 grams of the pure salt in 100 c.c. of distilled water.
3. Alum.
   9.5 grams of potassium alum, $Al_2(SO_4)_3K_2SO_4\ 24H_2O_4$ are dissolved in 100 c.c. of distilled water.
4. Sodium Carbonate.
   8.6 grams of Na_2CO_3 are dissolved in 100 c.c. of distilled water.
5. Acetic Acid, 2 N.
   12 grams of glacial acetic acid are made up to 100 c.c. with distilled water.

PROCEDURE:

10 c.c., or preferably 10 grams of blood to which hirudin has been added to prevent clotting, are placed in a 250 c.c. Erlenmeyer flask and 90 c.c. of distilled water and 3 drops of 2 N acetic acid added. The blood solution is then slowly warmed on a water bath which is finally allowed to boil for half an hour. The precipitated albumin, which must be chocolate brown in color (i.e., showing no tinge of red,) is cooled by tap water, and filtered with suction on a Büchner funnel, being then thoroughly washed with boiling hot distilled water until the filtrate gives no chloride

test with N/10 $AgNO_3$ (*). The filtrate should be clear and of a pale red color. It is transferred to a 500 c.c. Erlenmeyer flask and 12 drops of the potassium alum solution and 10 drops of the sodium carbonate solution added. The solution is then boiled for several minutes and filtered hot through a fluted filter into a 500 c.c. graduate. The filtrate must now be clear and quite colorless. Wash the filter paper with hot distilled water until the volume is nearly 250 c.c. and then transfer to a shallow porcelain evaporating dish. Add 2.5 c.c. of the indicator and titrate the yellow solution with the silver nitrate solution until a faint *permanent* red color is produced, stirring constantly during the titration. The duplicate can be made more accurate by removing the red color in the first titration by adding a pinch of sodium chloride or a drop or two of a chloride solution, and using the first solution for comparison. This solution will have a pure yellow color with a white silver chloride precipitate equal to that obtained in the second titration. It is well, however, to reserve a half portion of the original solution in order to match the shade of red.

If the solution is acid, it must be neutralized with sodium carbonate.

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* The washing can be further checked, if desired, by igniting the precipitate and paper in a covered platinum crucible until carbonized and volatile matter expelled, lixivating several times with hot water, filtering and testing the filtrate for chlorides. No turibidity should occur on adding N/10 Ag NO_3.

THE PREPARATION OF CAPRYLIC ALCOHOL* FROM CASTOR OIL FOR USE AS A FOAMING INHIBITOR IN MICROCHEMICAL ANALYSIS

O. I. Lee, B.S.

Castor oil is composed chiefly of triricinolein, the glyceride of the unsaturated hydroxylated aliphatic acid, ricinoleic, $CH_3(CH_2)_5CH(OH).CH:CH(CH_2)_8.COOH$[1], although small amounts of sebacin, tristearin, and the glyceride of dihydroxystearic acid are known also to be present. When destructively distilled in a good vacuum, it breaks up into heptaldehyde (oenanthal), undecylenic acid and acrolein, together with some heptoic acid, possibly as follows:

$$CH_3(CH_2)_5.CH(O\ H).CH:CH(CH_2)_8.CO\ CH_2O$$

$$CH_3(CH_2)_5.CH(O\ H).CH:CH(CH_2)_8.\dot{C}O.C.OH+O_2=$$

$$CH_3(CH_2)_5.CH(O\ H).CH:CH(CH_2)_8.\dot{C}O.CH_2O$$

ricinolein

$$3CH_3(CH_2)_5.CHO+3CH_2:CH(CH_2)_8.COOH+CH_2:CH\ CHO$$

heptaldehyde undecylenic acid acrolein

It is necessary to assume that oxygen is supplied, but since the temperature is high (300 C. to 320 C.), and since heptoic acid is known to be formed (by oxidation of the heptaldehyde) this assumption is not unreasonable. Possibly it is obtained at the expense of part of the oil itself. The insoluble, bulky, spongy, india-rubber-like residue appears to be the anhydride of triundecylenic acid, $(C_{11}H_{20}O_2)_3.H_2O$[2].

If the undecylenic acid thus made is separated and fused with caustic potash, the molecule is ruptured at the double bond, hydrogen is evolved, and potassium oxalate and potassium pelargonate remain in the fusion, from which upon acidification and subsequent steam distillation, the pelargonic (nonylic) acid

* Methyl-n-hexylcarbinol, secondary octylalcohol, octanol (2).

may be recovered[3]. The reaction may be represented:

$CH_2:CH(CH_2)_8.COOH + KOH = CH_2:CH(CH_2)_8.COOK + H_2O$

undecylenic acid — potassium hydroxide — potassium undecylenate

$CH_2:CH(CH_2)_8.COOK + 2\,KOH + 2\,H_2O$

potassium undecylenate — potassium hydroxide

$= CH_3(CH_2)_7.COOK + COOK.COOK + 4H_2$

potassium pelargonate — potassium oxalate — hydrogen

Treatment with alkali tends to shift a double bond, if possible, nearer to the carboxylic group; and it will be observed that such a molecular transformation must have occurred in this case before the linkage was finally broken.

When ricinoleic acid is fused with caustic potash, however, merely a simple schism takes place, hydrogen is evolved and methyl-n-hexylcarbinol and potassium sebacate, a homologue of potassium oxalate, are formed:

$CH_3(CH_2)_5.CH(OH).CH:CH(CH_2)_8\,COOH =$

$\qquad\qquad\qquad\qquad\qquad\qquad\quad H\quad OK$

$\qquad\qquad\qquad\qquad\qquad\qquad\quad H\quad OK$

ricinoleic acid — potassium hydroxide

$CH_3(CH_2)_5\,CH(OH).CH_3 + KOOC(CH_2)_8.COOK + H_2$

methyl-n-hexyl-carbinol — potassium sebacate — hydrogen

Similarly, with ricinolein on fusion with caustic alkali[4]

$CH_3(CH_2)_5.CH(O\quad H).CH:CH(CH_2)_8.CO.CH_2O$

$CH_3(CH_2)_5.CH(O\quad H).CH:CH(CH_2)_8.CO.C.OH + 3NaOH =$

$CH_3(CH_2)_5.CH(O\quad H).CH:CH(CH_2)_8.CO.CH_2O$

ricinolein

$3CH_3(CH_2)_5CH(OH).CH:CH(CH_2)_8.COONa +$

sodium ricinoleate — $CH_2OH.CHOH.CH_2OH$ glycerol

$3CH_3(CH_2)_5CH(OH)CH:CH(CH_2)_8.CO.ONa + 3NaOH + 3\,H_2O =$

sodium ricinoleate — sodium hydroxide

$3CH_3(CH_2)_5.CH(OH).CH_3 + 3NaO.OC(CH_2)_8CO.ONa + 3\,H_2$

methyl-n-hexyl-carbinol — sodium sebacate — hydrogen

In making the caprylic alcohol for laboratory use, an ordinary tin can with a conical top may be utilized very successfully for working up small quantities, increasing the capacity of the can used one gallon for every kilo of castor oil taken, since there is considerable frothing during the first part of the reaction. For the best yields, however, an iron still is preferable, as the temperature may then be carried higher and the castor oil soap completely decomposed. In order to avoid charring at the higher temperatures, and, in the case of the tin cans, to prevent oxidation of the tinned iron, the heat should be diffused. To this end, the bottom of the still may be protected with a sheet metal baffle plate. Towards the last, the temperature is high enough to melt the solder on the tin cans, but the alkaline soap that at first finds its way out seems to form an infusible cement with the solder and effectively prevents any further leakage.

To 933 grams of castor oil contained in a gallon tin can is added a solution of 240 grams of sodium hydroxide in 150 c.c. of water. A Liebig condenser is connected as a reflux, and a metal ball condenser, superposed on this, connected with a glass tube leading down to an hydraulic trough. All connections should be gas tight. Upon heating the alkali and castor oil soap to boiling, a steady stream of hydrogen proceeds from the end of the glass tube dipping under water, and after the air has been expelled from the system, a test tube filled by displacement will be found to burn freely with a pale colorless flame. This evolution of gas is so voluminous during the early stages of the reaction that much caprylic alcohol is swept out as a white sweet-smelling smoke if the Liebig condenser is not reinforced by a ball condenser. The heating is continued as long as there is any marked bubbling of hydrogen. The glass condenser is then inclined to the still and connected to it by a goose-neck, and the heating resumed. The caprylic alcohol, mixed with the products of secondary reactions, slowly distils over with the water on which it floats. The first oily drops are white and very fragrant (methyl-n-hexyl ketone ?), but the oil is soon tinged yellowish and acquires a more fatty odor. Later it becomes darker and displays a greenish fluorescence (hydrocarbons). The oil may be separated from the water from time to time, and the water returned to the still to aid the distillation. To the same purpose, it is advisable to insulate

the top and sides of the still with asbestos cardboard. If much more water than is originally taken is added, excessive foaming is likely to occur. The heating is continued as long as any crude oil goes over. The collected oil is then fractionally distilled under atmosphoric pressure, cooling with the inner tube of a separable Liebig condenser. The first fractions may be taken as follows:

No. 1	No. 2	No. 3
100 C. to 170 C.	170 C. to 181 C.	181 C. to 200 C.

The water is then separated from No. 1, and it is combined with No. 2 and No. 3, redistilled, and the fraction (No. 4) from 170 C. to 181 C. reserved for use as caprylic alcohol. Most of this boils at 178 C. Better results and a purer product may be obtained with the aid of a fractionating tower, but since the impurities present in the upper and lower fractions are chiefly inert ketones, ethers, and hydrocarbons, it is not necessary to obtain a chemically pure product for use as a foaming inhibitor. The freshly distilled caprylic alcohol made in this manner is of a pale straw color which darkens to yellow with age.

The pure alcohol boils at 179.5 C.[5] The specific gravity is 0.8193 at 20 C./4 C.[6]

The yield of crude oil from 933 grams of castor oil is 258 grams. From this, 107 grams of caprylic alcohol (No. 4) was recovered, corresponding to 11.5 per cent. of the weight of castor oil taken or 27.5 per cent. of theory. The yields are somewhat better with larger quantities.

Attention is also directed to the fact that methyl-n-hexyl-carbinol may be conveniently made from the heptaldehyde obtained by the vacuum distillation of castor oil, by means of the Grignard reaction:

$$CH_3(CH_2)_5CHO + CH_3MgI = CH_3(CH_2)_5CH(OMgI)CH_3$$

$$CH_3(CH_2)_5CH(OMgI)CH_3 + HOH = CH_3(CH_2)_5.CH(OH)CH_3 + IMg.OH$$

The yield is good and the product water-white and sweet smelling.

Preparation of Sebacic Acid[7]

The alkaline residue remaining in the still is boiled with considerable water and filtered hot with suction through a large Büchner funnel fitted with three filter papers. The clear dark brownish yellow filtrate having a greenish fluorescence is cooled

and extracted twice with ether. The extract is rejected, and the alkaline water solution poured into a large porcelain evaporating dish and slowly neutralized with the calculated amount of diluted sulphuric acid (about 160 c.c. of conc. H_2SO_4 if 240 gm. of sodium hydrate be used). The foaming can be kept down by adding a little ether from time to time. After acidification and thorough agitation, the whole is transferred to a six or eight liter flask and heated over live steam. The fatty acids gradually collect on top in a dark brown layer. The contents of the flask are then vigorously shaken and allowed to cool to room temperature. The layer of brown acids on top will again solidify, but a large quantity of minute white pearly crystals separates underneath. By tipping the flask, these may be run out from underneath the cake of impure acid into a porcelain evaporating dish, and the small fragments of the caked acids removed. The white crystals may then be filtered on a Büchner funnel without contamination. The residual acid cake is boiled with fresh water and cooled in the ice box. Since the sodium sulphate and sodium oxalate have now been eliminated, the second yield of acid is much greater than the first. It is removed in the same manner and added to the preceding precipitate and the process continued until the floating fatty acids are pasty or liquid when cold.

The crude sebacic acid thus obtained, when washed and dried, titrated within 1.7 per cent. of the theoretical value for $HO.OC.(CH_2)_8.CO.OH$. A sample recrystallized from acetone melted at 128 C. (uncorrected), the melting point of the pure acid being 133 C.

Yield, about 8.7 per cent. of weight of castor oil taken or 13.4 per cent. of theory.

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[1]Krafft. Berichte d. Deutsch. chem. Gesellsch., xxi, 2730.
Kasansky. Jour. f. prakt. Chemie, 1900, lxii, 363.
[2]Fendler and Thoms. Archiv. d. Pharm., 1901, 1, (239)
[3]Krafft. Berichte d. deutsch. chem. Gesellsch., 1877, x, 2034
Becker. Berichte d. deutsch. chem. Gesellsch., 1878, xi, 1413.
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[4]Moschnin. Ann. d. Chemie u. Pharmazie, 1853, lxxxvii, 111.
Bouis. Ann. d. Chemie u. Pharmazie, 1856, xcvii, 34.
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Nelson. Jour. Chem. Soc., 1874, (2), 12, 301, 507, 537.

M. Freund u. F. Schönfeld. Berichte d. deutsch. chem. Gesellsch., 1891, xxiv, 3351.
[5]Schorlemmer. Jahresb. d. Chemie, 1875, 285.
[6]Brüll. Ann. d. Chemie u. Pharmazie, 1880, cciii, 28.
[7]Bouis. Ann. d. Chemie u. Pharmazie, 1851, lxxx, 303; 1856, xcvii, 34.
Witt. Berichte d. deutsch. chem. Gesellsch. 1874, vii, 220.
Nelson. Jour. Chem. Soc., 1874, (2), 12, 301, 507, 537.

NOTE ON AN IMPROVED INHIBITOR OF FOAMING FOR USE IN THE MICROCHEMICAL ANALYSIS OF BLOOD AND URINE

O. I. Lee, B.S.

Although in the past kerosene, albolene, amyl alcohol and caprylic alcohol* have all been recommended to obviate the annoying foaming incident to the rapid aspiration of ammonia from blood and urine solutions, it has been found in practice that even the last, which is the most effective, cannot be absolutely depended upon to exercise its proper function. Certain specimens of blood, particularly those high in dextrose, are apt to bubble over after about twenty minutes running in the urease method of determining urea nitrogen, vitiating the results, for it is impracticable to introduce more of the foam inhibitor, because of the danger of back pressure and of loss of ammonia. Moreover, the cost of the caprylic alcohol becomes an appreciable item of expense where numerous routine determinations are made, and the compound is sometimes not available. A series of empirical experiments were accordingly carried out in the search for a more satisfactory substitute. Such inert and high boiling (essential) oils as cineol (eucalyptol), methyl para cresol, oenanthic ether (ethyl heptylate), linaloöl, terpineol and diphenyl oxide, were tried out, alone and in combinations with each other and with amyl alcohol, and it was found eventually that a mixture of 30 per cent. by volume of amyl alcohol with 70 per cent. by volume of phenyl oxide gave excellent results, ten drops being sufficient to prevent foaming for an indefinite period in the usual amounts of blood and urine taken. In making the mixture, it was found that the amyl alcohol must be added to the diphenyl ether, and not vice versa, otherwise the solution will be turbid and will have to be boiled, cooled and filtered through a dry filter paper.

* The commercial article sold as "caprylic alcohol" is chiefly methylnormal-hexyl-carbinol and boils at 179.5 C.

Diphenyl oxide, phenyl oxide, diphenyl ether, or phenyl ether, as it is variously known, is a colorless oil melting at 27 C., boiling at 259 C., and having a specific gravity of 1.07. It is a by-product in the manufacture of carbolic acid from monochlorbenzene when heated at a high pressure and temperature with potassium hydroxide:

$$\underset{\text{Chlor benzene}}{C_6H_5Cl} + KOH = \underset{\text{phenol}}{C_6H_5OH} + KCl$$

$$\underset{\text{Chlor benzene}}{2\ C_6H_5Cl} + 2\,KOH = \underset{\text{diphenyl ether}}{C_6H_5OC_6H_5} + 2\,KCl + H_2O$$

It has an odor recalling geranium leaves and may be procured from most dealers in essential oils.*

It is hoped that the above information will prove generally useful in altogether eliminating one of the troublesome elements encountered in carrying out procedures calling for the aëration of liquids having a tendency to foam.

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* George Lueders & Co., 218-220 Pearl St., New York, supply diphenyl oxide at about $2.75 per pound, under the name of "geranium artificial.

A SIMPLIFIED PROCEDURE FOR THE PREPARATION OF PARAPHENYLENEDIMETHYLDIAMINE SULPHATE. (OXIDASE STAIN).

(dimethylparaphenylenediamine or paramidodimethylaniline sulphate).

1: $4C_6H_4(NH_2)(N(CH_3)_2) \cdot H_2SO_4$

O. I. Lee, B.S.

The four methods recorded for the synthesis of this useful compound are as follows:

METHOD I. Formed together with dichloroparaphenylenediamine and dichloroparaphenylenedimethyldiamine by boiling paranitrosodimethylaniline with hydrochloric acid (Sp. gr. 1.2).

Richard Mohlan, B.19[2], 2010 (1886); Über die Einwirkung concentrirter Salzsäure auf Nitrosodimethylanilin.

METHOD II. By the reduction of mononitrodimethylaniline with tin and hydrochloric acid.

Adolph Weber, B. 8[1], 715 (1875); Über Derivate des Dimethylanilins.

METHOD III. By the reduction of sodium paradimethylaminoazobenzene-sulphonate (methyl orange, helianthine,) with ammonium sulphide or stannous chloride.

Methyl orange is decomposed, like the majority of azo-compounds, by stannous chloride in hydrochloric acid into two molecules, produced by the addition of hydrogen to the double-linked nitrogen atoms:

$$HSO_3.C_6H_4.N{:}\ N.C_6H_4.N(CH_3)_2 + 2SnCl_2 + 4HCl =$$
Helianthine*

$$HSO_3.C_6H_4.NH_2 + H_2N.C_6H_4.N(CH_3)_2 + 2SnCl_4$$
Sulphanilic Acid Paramidodimethylaniline.

Emil Fischer, B. 16², 2235 (1883); Bildung von Methylenblau als Reaktion auf Schwefelwasserstoff.

Practical Organic Chemistry, Sudborough and James, Experiment 166, p. 208.

METHOD IV. By diazotizing dimethylaniline and reduction of the paranitrosodimethylaniline and of $(C_6H_4N(CH_3)_2)_2N_2O$ by tin and hydrochloric acid.

$$C_6H_5N(CH_3)_2.HCl + HNO_2 = (NO)C_6H_4N(CH_3)_2.HCl + H_2O$$
Dimethylaniline Hydrochloride — Nitrous acid — Paranitroso-dimethylaniline Hydrochloride

$$(NO)C_6H_4N(CH_3)_2.HCl + 4H = NH_2.C_6H_4N(CH_3)_2.HCl + H_2O$$
paramido-dimethylaniline Hydrochloride

Conrad Schraube, B. 8¹, 616 (1875); Über Nitrosodimethylanilin.

Adolph Weber, B. 10¹, 761 (1877); Über Derivate des Dimethylanilins.

C. Wurster, B. 12¹, 522 (1879); Über die Methylderivativ des Paraphenylenediamins.

Discussion of Methods

The first method is merely of academic interest, the yield being poor and difficulties attendant upon the separation of the closely allied products of secondary reactions.

Little attention, either, seems to have been paid to Weber's observation concerning the reduction of mononitrodimethylaniline. He obtained a substance which gave an intense reddish violet coloration with the weakest oxidation agents (ferric chloride), and which he concluded from a chlorine determination: of the hydrochloride, was amidodimethylaniline.

In the third method, since the highest relative yield of diamine

* The dye is the sodium salt of the acid whose formula is given.

from helianthine theoretically possible is only 41.6% as compared with 112.4% from dimethylaniline, and since their relative costs are about as 8 : 3, free base for oxidase stain prepared from the dye is more than seven times as expensive as that made from dimethylaniline.

According to Schraube, in the fourth method, paranitrosodimethylaniline hydrochloride treated with tin and hydrochloric acid dissolves to a colorless liquid, colorless crystals of an organic tin compound precipitating simultaneously, part of which remains in solution. This compound is isolated, and dissolved in water, and hydrogen sulphide passed until the tin is completely precipitated, whereupon excessively hygroscopic crystals of paraphenylenedimethyldiamine hydrochloride are obtained following evaporation and cooling of the thick syrup. He obtained the free base by decomposing a concentrated water solution of the hydrochloride with a saturated solution of sodium carbonate and subsequent extraction with ether and evaporation in a vacuum.

Wurster elaborated and improved the method, but does not insist on filtering the (troublesome) tin compound although he deems it more expedient to do so, and remarks that by saturating the mother liquor with gaseous hydrochloric acid more of the double salt will appear after a time.

The fluid poured off from the residual tin after reduction is poured directly into sodium hydroxide solution, and in the case of the tin double salt, he recommends introducing it into the solution rather than pouring the caustic alkali over the same, adding that if the right conditions have been observed, all the tin will dissolve in the alkaline fluid and the base will float as a brownish oil on the warm liquor and can be so separated or with the aid of benzol. He admits, however, that the separated base frequently envelops part of the tin compound and forms with it thick, sticky, putty-like lumps which fall to the bottom. Nothing can be done, in this event, but repeatedly to extract the whole mass with ether and treat the pasty material on a filter with warm water. The base dissolves, the lumps fall apart, and from the water solution, the base can be precipitated with concentrated sodium hydroxide and extracted with benzol or ether.

If the free base or its solution in benzol or ether is dried with calcium chloride, the temperature will rise quickly to 250 C. after

the solvent has been distilled and it distills at 250 C.—257 C. as a pale yellow colored oil which tends to freeze in the condenser tube. The crude product is contaminated by traces of a fluid body. If this is removed by pressing and the base recrystallized from benzol and ligroin according to the directions given, an absolutely pure compound may be obtained which it is claimed will not discolor and assume the pale reddish or violet tint that crystals in an impure condition do.

The average yield is stated as about 58% of the theoretical.

These methods of Schraube and Wurster, starting from the cheap and easily procured intermediate product dimethylaniline, were carefully repeated several times as directed, but in no case were colorless crystals of any tin compound other than stannous chloride obtained. Ultimately, however, from the experience gained, a procedure was evolved which, among other advantages, does not entail the trouble and loss incident to the separation and decomposition of an organic tin compound, which omits any distillation of the free base, and which gives an excellent yield of a very pure product, the purification being rapidly effected by the fractional precipitation of the sulphate by alcohol and ether, after Fischer, instead of by the tedious benzol-ligroin method.

Preparation of Paranitrosodimethylaniline Hydrochloride

Paranitrosodimethylaniline hydrochloride is first prepared, various procedures being given, but that of Cain and Thorpe* is very satisfactory and insures a good yield.

100 grams dimethylaniline,
400 grams concentrated hydrochloric acid (345 c.c.)
60 grams sodium nitrite (74 gm. KNO_2)

The dimethylaniline is dissolved in the hydrochloric acid, and ice added till the temperature sinks below 0 C. (—9 C.). The sodium nitrite, dissolved in a small quantity of water, is *slowly* added. the whole being stirred mechanically and the temperature not being allowed to rise above 8 C. (use plenty of ice and salt.) The hydrochloride of p-nitrosodimethylaniline separates out as an orange yellow precipitate, and after standing half an hour is filtered at the pump on a Büchner funnel, well drained and pressed,

* The Synthetic Dyestuffs and their Intermediate Products, p. 205.

washed with a little dilute hydrochloric acid, thoroughly pressed between two large sheets of filter paper, and dried in the air.

It can be recrystallized from hot water, but decomposes if the solution is heated to boiling.

Properties: Yellow needles; M. P. 177°.

It is not essential to start with a chemically pure dimethylaniline, although perhaps preferable, since monomethylaniline which may be present is contained for the most part in the mother liquor as nitrosamine.

Simplified Procedure for the Preparation of Paraphenylenedimethyldiamine

100 gm. paranitrosodimethylaniline hydrochloride are dissolved in the least amount of water in an Erlenmeyer flask, 250 gm. of mossy tin and 500 c.c. of concentrated hydrochloric acid added, a funnel covered with a watch glass inserted, and the whole heated on a water bath until the tin is completely dissolved. The solution will never become colorless, but will eventually be pale yellow. It is practically impossible to extract the free base at this point by addition of excess of strong alkali, since the sodium stannite formed seems to have an infinite capacity for forming an inseparable emulsion with ether. Benzol is objectionable because its higher boiling point necessitates a longer exposure and more deleterious oxidation of the base during evaporation. The solution is accordingly diluted with several volumes of water and hydrogen sulphide passed in to saturation. The stannous sulphide is filtered off on a large Büchner funnel, and washed with a little hot water, and hydrogen sulphide passed into the filtrate. There should be little or no further precipitation. Besides removing a large amount of the tin present, the solution is thus saturated with a reducing gas which tends to inhibit oxidation of the sensitive base in subsequent operations. The concentration of acid is too great to make it feasible to remove all the tin in this way, and diluting the solution more would require an excessive amount of ether for extraction. Accordingly, the major portion of the remaining tin is precipitated as stannous hydroxide by the cautious addition of 30% sodium hydroxide solution, but taking care not to add enough to set the base free. This point can be determined by watching for a faint pink or violet color,

and it is desirable, though not necessary, to stop just short of its permanent appearance. In any event, the solution must be acid. The bulky white precipitate is filtered on a large Büchner funnel, washed with a little hot water, and the cloudy filtrate cooled, saturated with ether and extracted three times with decreasing volumes of ether after the addition of enough cold 50% caustic soda solution to insure the solution of the remaining tin as sodium stannite after its precipitation as stannous hydroxide. The ethereal extract is filtered through a dry filter paper into an Erlenmeyer flask and enough granular calcium chloride added to cover the bottom of the flask which is corked and allowed to stand in a cool dark place for at least 24 hours, longer if convenient. The calcium chloride absorbs not only water and alcohol, but much of the foreign coloring matters, and if allowed to stand long enough, the ether solution becomes a clear ruby red. The solution is carefully decanted into a tall beaker large enough to take care of any foaming which is apt to occur, (it is safer to add it in portions,) covered with a watch glass, and evaporated to a small volume on a water bath. Enough alcohol is added to the free base to make it mobile, the solution well chilled, and cold 50% sulphuric acid dissolved in an equal volume of alcohol slowly added with constant stirring and occasional cooling, until no further precipitation of sulphate occurs and the solution is acid. Care must be taken not to add too much acid. Several volumes of ether are added, the whole well mixed and the crude precipitated paraphenylenedimethyldiamine sulphate filtered on a Büchner funnel, pressed down with a porcelain spatula, and washed with small quantities of ether. A little more sulphate of inferior quality can usually be recovered from the filtrate by adding several volumes of alcohol and saturating with ether, but should not be united with the first precipitate until purified at least once as described in the following procedure for

Purification of Crude Paraphenylenedimethyldiamine Sulphate

The crude yellowish green sulphate is completely dissolved in the minimum quantity of cold water, an equal volume of alcohol added, and then several volumes of ether. If the last separates, enough alcohol is added to dissolve it. The precipitated sulphate is filtered as before, pressed, and well washed with ether. That

remaining in the filtrate may be recovered by displacement with more alcohol and ether. If the main precipitate still shows a tinge of yellow, or greenish yellow, the operation should be repeated until a pale cream colored precipitate is obtained. The filter paper and contents is then blown into a porcelain evaporating dish, quickly transferred to an oven at 100° and dried for 15 minutes. After removing any greenish colored particles which may be found around the edge, the compound is thoroughly ground in a porcelain mortar and dried for an hour at the same temperature. The final product should be of a very faint buff color and should not turn pink or lavender on exposure.

The average yield is about 79% of diamine base calculated from dimethylaniline, or 89% of the amount taken.

Properties of the Diamine Base (*Wurster.*)

Crude dimethylparaphenylenediamine melts at 38-39°, but by repeated crystallizations the melting point rises to 41°. The pure base boils quite unchanged at 257° (the stem of the thermometer being completely immersed in the vapor.) The distillate is at first colorless, but since the air quickly changes the warm fluid base to a yellowish color, it is advisable to conduct the distillation in a stream of hydrogen.

The base recovered from saturated solutions forms long white needles; by evaporating the solution in ligroin, thick short prisms are obtained with developed bases. The molten and strongly heated base, although having an extraordinary capability for crystallizing, easily shows the phenomenon of super-melting. On this account it is advantageous in crystallizing from solutions as well as in the case of the fluid base, always to add a crystal to seed it. This effects in all cases a quick solidification.

The base is easily soluble in cold water, very easily soluble in alcohol, benzol, chloroform, etc., somewhat less so in ether, with difficulty in ligroin. With steam, it goes over in small but distinctly appreciable quantities.

Properties of Paraphenylenedimethyldiamine Salts

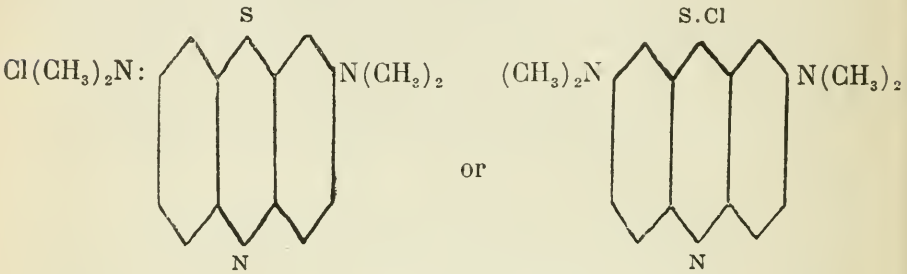
The salts of paradimethylphenylenediamine are easily soluble in water, but only slightly soluble in alcohol. The sulphuric acid salt can be obtained from the hot saturated water solution fre-

quently in fine white leaflets, is somewhat soluble in alcohol, but insoluble in ether.

PbO_2 and aqueous HOAc give a magenta red solution changing to violet black.*

Colors wood and paper made from wood, crimson.**

H_2S and an oxidizing agent (e.g. $FeCl_3$ or H_2O_2) gives methylene blue, a salt of tetramethylthiodiphenylamine.



Methylene Blue

The formation of methylene blue affords the most delicate and certain reaction for the detection of hydrogen sulphide in neutral or acid solution, far exceeding in this respect the reactions with lead salts and nitroprussides. 100 c.c. of the liquid to be tested should be treated with 2 c.c. of fuming hydrochloric acid, and a few grains of dimethylparaphenylenediamine sulphate added. On then adding a drop or two of ferric chloride solution, methylene blue will be formed either immediately or on standing, if any hydrogen sulphide was previously present.

It has been demonstrated by Fischer that the presence of one part of hydrogen sulphide in more than 50,000,000 can be detected by this method.

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* Lauth, C. R. III, 975.
** Wurster, B. 20, 808.

THE DETERMINATION OF TOTAL IRON IN URINE

O. I. LEE, B. S.

Dissolve 10 gm. of ammonium persulphate in 25 c.c. of cold distilled water, add 10 c.c. of concentrated NH_4OH and pour the solution into 100 c.c. of the urine contained in a beaker. Boil for four minutes, cool to room temperature and filter on an ashless filter paper. *Wash the precipitate thoroughly with hot distilled water until the filtrate gives no turbidity with a few drops of* N/10 $AgNO_3$. Ignite paper and precipitate in a platinum crucible (previously cleaned with hot conc. HCl,) cool, add 2 c.c. of 50% HCl (*Fe free,*) and warm for several minutes. Make up volume to 10 c.c. in a 25 c.c. graduate and add 5 c.c. of a filtered saturated solution of potassium thiocyanate, KCNS (*Fe free.*)

To another 25 c.c. graduate, add 2 c.c. of 50% HCl (Fe free,) 5 c.c. of saturated KCNS solution, and make up to 15 c.c. The solution should be clear and colorless. Place both graduates on white paper in a good light and titrate the latter (blank) solution immediately with a standard iron solution of $FeCl_3$ containing 1/10 mmg. of Fe per c.c., until the depths of color agree.

Normal urine runs from 0.06 mg. to 0.12 mg. of Fe per 100 c.c.

A SIMPLE PROCEDURE FOR THE PREPARATION OF COLLOIDAL GOLD FOR DIAGNOSTIC PURPOSES

O. I. Lee, B.S.

The first attempts to prepare colloidal gold in the St. Luke's laboratory were made according to the procedure recommended by Miller, Brush, Hammers, and Felton[1], but in spite of the greatest care the solutions obtained were uniformly poor. Eventually it was decided to experiment independently with a view of getting some insight into the trouble which we and many of our predecessors had experienced; and ultimately a reliable and exceedingly simple technique was evolved for preparing colloidal gold which will satisfy every clinical requirement.

The glassware used was the Pyrex brand. It was cleaned with no more attention than that usually given to laboratory apparatus and was rinsed only with tap water. Indeed, it was noticeable that solutions with which it came in contact did not wet the surface, but tended to form in drops. It is advisable, however, to avoid beakers that have bad scratches, as reduction tends to take place there.

Ordinary distilled water was used; no attention was paid to the time that had elapsed since distillation, and the water was drawn from stock containers. In view of the impurities possibly contained in some of the reagents*, and the known toleration of col-

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* Commercial formalin always contains formic acid. Ten c.c. of the formalin used required 1.1 c.c. of N/10 NaOH for neutralization (phenolphthalein).

The maximum limits of foreign substances in the Merck's Blue Label Reagent potassium carbonate used were stated to be

Heavy metals	.0000%
Chlorides (Cl)	.0020%
Sulphate (SO_3)	.0075%
Nitrates (N_2O_5)	.0800%
Potassium cyanide (CN)	.0480%
Sulphides (S)	.0200%
Sulphites (SO_2)	.0080%
Thiosulphates ($K_2S_2O_3$)	.0800%
Phosphates (P_2O_5)	.0040%
Silicates	.0000%
Aluminum (Al)	.0400%
Calcium (Ca)	.0010%

loidal gold solutions for electrolytes†, it is felt that in an emergency tap water could be employed, and satisfactory solutions have been made with it. All the reagents were obtained from Merck and Company. The potassium carbonate and oxalic acid were of the Blue Label Reagent quality, the formalin U. S. P., but there would seem to be no objection to using any good grade of chemicals convenient. The gold chloride was in sealed ampoules, and it is noteworthy that the weight of "gold chloride" contained therein varies very considerably*.

The solutions were of the following strengths:

Gold chloride acid.....1 per cent.
Potassium carbonate.....2 per cent.
Oxalic acid.....1 per cent.
Formaldehyde (37% CH_2O).....2.5 per cent.
(5 c.c. formalin made up with 200 c.c. water.)

The solutions should preferably be freshly made, although all but the last keep very well. The oxalic acid probably does not deteriorate at all, but the potassium carbonate slowly tends to dissolve its container and incorporate silicates; the gold chloride is easily reduced upon exposure to dust or light, and the diluted formalin undergoes marked changes from oxidation, polymerization, etc.

It was assumed that, since good solutions had been prepared with 10 c.c. of 1 per cent. $AuCl_3$ per litre, this amount might be considered not excessive, provided reduction was not carried too far. Keeping this constant, it was found that an increase of potassium carbonate tended to give violet and purple solutions. This was the experience of Miller, Brush, Hammers and Felton, and so the original amount of 7 c.c. was retained. These solutions, however, were invariably cloudy and more or less opaque. (Ten drops (0.43 to 0.58 c.c.) of oxalic acid, and not more than 5 c.c. of diluted formalin were used). The amount of oxalic acid was then gradually increased and a marked improvement was at once apparent. The solutions were very much more transparent and the minimum amount necessary to obtain the maximum trans-

† 1.7 c.c. of 1 per cent. NaCl is necessary to precipitate completely 5 c.c. of colloidal gold in one hour. Five c.c. of colloidal gold will remain unaffected by 1 c.c. of 0.4 per cent. NaCl for an indefinite period.

* The contents of two tubes labelled as containing 15 grains (972 mg.) of "Gold Chloride Merck Acid" contained respectively 824 and 869 mg.

parency seemed to be about 1.75 c.c. This was also in accordance with the results obtained at Johns Hopkins, but the statement that "a large amount of oxalic acid without the subsequent addition of formaldehyde made clear satt rot colloidal gold solutions" could not be verified. The solutions obtained in this laboratory were beautifully clear, but always blue or purple, although the conditions were varied within wide limits.

Finally, the formaldehyde factor was studied. It was evident from the behavior of the oxalic acid not only that the formaldehyde was essential to secure the ruby red color, but that it was responsible for the brown turbidity*. Beginning, then, with 5 c.c., the amount was slowly reduced (1.75 c.c. of oxalic acid being used) and although for a long time no change could be noted in the appearance of the solutions, this very fact was proof that too much formaldehyde was being employed. Very suddenly, though, almost ideal solutions resulted, which moreover responded perfectly to known paretic spinal fluids. Flesch notes that he could not obtain good solutions unless he used much less than 10 c.c. of 1 per cent. formalin, usually 2 to 3 c.c.[2]. The proper amount seems to be in the vicinity of 0.83 c.c. of 2.5 per cent. formalin containing 37.0 per cent. by weight of CH_2O, or 8.07 mg. of CH_2O per liter of colloidal gold made up.

In order, then, that the correct amount of formaldehyde should be used, it is necessary to determine the value of the formalin. This may be conveniently done by a modification of the iodometric method of Ronijn[3], which we have worked out as follows:

Evaluation of Formalin

Ten c.c. of the formalin is made up to one liter with distilled water and 5 c.c. of this solution is mixed with 25 c.c. of decinormal iodine. 2N sodium hydroxide is added drop by drop until

* It is suggested that the brownish turbidity is caused by a suspension of reduced gold of almost molecular dimensions. Solutions which are fogged invariably deposit a brown precipitate transmitting violet light and resembling Purple of Cassius and the violet gold precipitated from colloidal gold solutions by electrolytes. Such particles may have been caused by reduction by trioxymethylene (paraformaldehyde), and we have observed that when the Ostwald pipette used in measuring the formaldehyde is allowed to remain on the desk several hours, an amorphous white precipitate can be seen in the tip, and if the pipette is used a very cloudy solution results, whereas a clean dry pipette gives clear solutions.

the liquid becomes clear yellow. After ten minutes, 2 c.c. of concentrated hydrochloric acid are added and the free iodine titrated with decinormal sodium thiosulphate. Two atoms of iodine are equivalent to one molecule of formaldehyde. If T equals the titration and S the specific gravity of the formalin, then

$$\text{Per cent. of } CH_2O \text{ equals } \frac{75 - 3\,T}{S}$$

Up to this point the gold and alkali had been added at 60 C., the oxalic acid at 80 C., and the formalin at 90 C. The experiment was made of introducing all the chemicals initially, keeping the temperature just high enough to cause gradual reduction. The results were eminently satisfactory, and we have since been guided entirely by the color changes, rather than by any definite temperature. This preliminary mixing of all the units of the reacting system insures their perfect and uniform distributon and eliminates the unfortunate and unavoidable local reduction effect entailed by the seriatim method of introduction of the reducing agents.

The following procedure has been finally adopted, and although it is not guaranteed to be infallible in giving *ideal* solutions, with a little experience it will be found not at all difficult to get 90 per cent. of the trials to answer every clinical requirement. As the cost of the chemicals is less than five cents per liter, and a dozen solutions may be prepared in an afternoon, it is no extravagance to discard even 50 per cent. of these should this be necessary.

Technique of Securing One Liter of "Satt Rot" Colloidal Gold

To 1 liter of distilled water in a clean liter flask add 10 c.c. of 1 per cent. gold chloride, 7 c.c. of 2 per cent. potassium carbonate, 1.75 c.c. of 1 per cent. oxalic acid, and 0.83 c.c. of 1:40 formalin (37.0% CH_2O) or equivalent amount of 8.07 mg. of CH_2O per liter. Mix thoroughly, cover with a watch glass, turn the burner on full, and heat with occasional stirring to between 80 C and 85 C. Then reduce the flame enough to keep the temperature constant. The wire gauze supporting the flask should be covered with a sheet of asbestos cardboard to prevent local reduction on that portion of the flask directly over the flame. The solution will

slowly develop through a series of colors* substantially as given below, though some of the shades are much more transient than others. The associated temperatures are merely typical and were recorded for a development which lasted about an hour.†

Color	Temperature
Faint blue green	65 C.
Pale blue green	74 C.
Pale bluish lavender	78 C.
Bluish lavender	79 C.
Pinkish lavender	80 C.
Bluish pink	82 C.
Pale pink	83 C.
Pink	84 C.
Deep pink	84 C.
Violet red	84 C.
Bluish red	83 C.
Deep bluish red	83 C.
Deep ruby red	83 C.
Dark ruby red (violet red in test tube or pipette)	83 C.
Rebound or color transition	83 C.
Orange red (orange pink in test tube or pipette)	83 C.

When the solution reaches its maximum depth of color, a remarkable lightening and change of color occurs within the space of a few seconds, and *until this rebound and transition of color has taken place the solution will be worthless for clinical purposes.* After this point has been passed, the burner may be turned out and the solution allowed to cool to room temperature, although if wanted at once there is no objection to pouring it into a flask and cooling in running water.

If the solution is heavily clouded, pour it out and examine the flask for spots and streaks of reduced gold transmitting green, blue, or violet light. If noticeably present, add a little aqua

* For the probable significance of these colors see Ostwald, "A Handbook of Colloid-Chemistry," page 32.

† Kaplan observed that the color change comes gradually as a rule in good colloidal gold solutions: Ztschr. f. d. ges. Neurol. u. Psychiatrie, 1915, xxvii, 246.

regia*, cover with a watch glass and warm for a few minutes, then rinse thoroughly and repeat the experiment. A clear colloidal gold solution leaves a clean container.

If the solutions still continue to be turbid and show a strong brownish fluorescence, a series of half a dozen liters should be made with amounts of diluted formalin decreasing and increasing by 0.05 c.c. from that originally used, and notice taken in which direction improvement occurs. We have observed that too little formalin will cause cloudiness, as well as too much.

It is, perhaps, advisable to store the solution in the dark, but it is not necessary to have it cold, and so far as we know the solution once made will keep indefinitely, certainly for several months. Contrary to the generally accepted requirement, a slight brownish surface dispersion was found to make no difference in the practical application of the colloidal gold. The practice of Kafka[4] of rejecting all solutions which are tinged blue may be adopted; and it may also be stated for the guidance of those making colloidal gold that the correct color is duplicated almost exactly (in a test tube comparison) by a solution of 5 c.c. of 20 per cent., by weight of cobaltous nitrate ($Co(NO_3)_2\ 6H_2O$) plus 0.30 c.c. of 0.25 per cent., potassium dichromate ($K_2Cr_2O_7$) solution plus 10 c.c. of water.*

A certain amount of black sediment often accumulates on the bottom of the colloidal gold solution, but this does not impair its usefulness.

The procedure given above applies to the preparation of one liter of colloidal gold at a time, and it is not advisable to experiment with quantities greater or less than that amount until some experience has been gained.

None of the solutions prepared by this method were of the protected type, and all of them gave typical curves, not only with general paresis, but with lues, tabes, and meningitis. No attention was paid to the question as to whether the final solution was neutral or not, but 5 c.c. of every solution prepared was completely

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* Seven volumes of concentrated hydrochloric acid to two volumes of concentrated nitric acid.

* It is the intention to publish later the composition of a complete permanent standard colloidal gold color scale blended from inorganic constituents similar to the above, and based upon the colors observed with pathological and spinal fluids.

precipitated in one hour by 1.7 c.c. of 1 per cent. sodium chloride and no change of color whatever could be detected with normal spinal fluids.

In an article by Hulbert[5] the inherent disadvantages of formalin are discussed and a neat method is shown of avoiding these by utilizing formaldehyde vapor, oxalic acid being omitted, and the course of the reaction being guided by the color of the solution.

The tubes used for the clinical tests were heavy glass, lipless test tubes, measuring 11/16 to 3/4 inches x 6 inches. They are not readily broken in washing, and the point of the 1 c.c. pipette may safely be pressed against the bottom of the tube in order to avoid air bubbles and danger of spinal fluid being carried into the mouth of the operator. After using, they are rinsed in tap water and immersed overnight in a 1:10 dilution of aqua regia. This effectually dissolves any precipitated gold which may be sticking to the glass and is otherwise removed only with difficulty.

The 1 c.c. pipettes should be graduated to the tip, otherwise two measuring points must be determined and time lost by returning the amount in excess of 1 c.c. to the preceding tube. It is well to see, too, that there is a wide margin between the 1 c.c. mark and the top of the pipette.

Although in the usual technique the concentration of the spinal fluid in the tenth tube is 1:5120, the ratio of the total volume to the volume of the colloidal gold employed is higher than in the other tubes, unless 1 c.c. is removed from the tenth tube and rejected. This point is not clearly brought out in the descriptions of the technique which have come under our notice, with the result that the curves obtained before making this correction had an upward "kick" at their ends which was rather misleading.

Conclusions

1. That colloidal gold solutions which conform to every clinical requirement (differential paretic, luetic, tabetic, meningitic, and normal curves) may be made with comparative ease.

2. That it is quite unnecessary to use other than ordinary distilled water from any convenient laboratory still.

3. That nothing is to be gained by elaborate precautions in cleaning the glassware used, and that apparatus washed with

soap and water, rinsed in tap water, and hung up to dry has been found entirely satisfactory.

4. That the reagents used may all be introduced at the beginning of the experiment, and temperature other than the final one disregarded.

5. That providing sufficient oxalic acid is present, the only critical points involved are the amount of formaldehyde used*, and the passing of the color transition point at which the solution lightens and becomes orange red.

6. That solutions so prepared were of the proper color and transparency and precipitated properly with a dilute electrolyte.

7. That if a thick layer of the solution is transparent, and of the proper color, a certain amount of surface dispersion is no bar to its usefulness.

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* This is in the vicinity of 17 per cent. of that recommended by Miller, Brush, Hammers and Felton.

[1]Miller, Brush, Hammers and Felton. A Further Study of the Diagnosis Value of the Colloidal Gold Reaction, together with a Method for the Preparation of the Reagent. Bull. Johns Hopkins Hosp., 1915, xxvi, 298.

[2]Flesch. Ztschr. f. d. ges. Neurol. u. Psychiatrie, 1914, xxvi, 318.

[3]Ronijn. Zeit. Anal. Chem., 1897, xxxvi, 18.

[4]Kafka. Deutsch. med. Wchnschr., 1913, xxxix, 1874.

[5]Hulbert H. S. Technical Improvement in Lange's Colloidal Gold Test. Jour. Mich. Med. Soc., January, 1916.

LABORATORY TECHNIQUE*

THE METHODS EMPLOYED AT ST. LUKE'S HOSPITAL

F. C. Wood, Karl M. Vogel,
and
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With the extraordinary development of technical methods, the hospital laboratory to-day is called upon to perform work of an extremely complicated nature in much greater amount than was the case ten years ago. In fact, the total volume of work is so large that unless some definite system is employed mistakes are certain to occur. Moreover, it is important that the laboratory procedures be standard ones, that the limits of accuracy be thoroughly understood, and that the methods of carrying out the tests do not vary from year to year with the shifting of the laboratory internes or of the department chiefs; in this way only can the reports be of value to the attending physicians. For example, since there is considerable variation in the readings obtained with the Tallqvist, Sahli, and Fleischl-Miescher hemoglobinometers, it is necessary for diagnostic and therapeutic purposes that the clinician know with what apparatus a hemoglobin determination has been made. In a case of doubtful syphilis, also, it is very important that the physician understand whether the Wassermann test has been done according to the original method, by which a certain small percentage of late syphilides give no reaction, or with one of the highly sensitive cholesterinized antigens, by which a faint reaction may possibly be obtained in a person not afflicted with the disease.

In attempting, therefore, to standardize the laboratory work in the Pathological Department of St. Luke's Hospital, there was prepared some time ago a series of small typewritten notebooks, containing the necessary directions for the examination of blood, urine, sputum, etc., methods of preparation of pathological material, and the simpler bacteriological and serological procedures. In the course of time these notebooks have been

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expanded; and, as interest in the technical methods used at St. Luke's has been expressed by workers in other hospitals, it has been felt that perhaps a wider circle can be reached by the publication of our laboratory procedures in the Hospital Report. While the directions given are of value chiefly to the interne and to the recently graduated physicians, who are apt to be the persons doing most of the laboratory work in connection with patients, it is hoped that they may also prove a useful guide for older practitioners whose active participation in laboratory work is only occasional.

Inasmuch as these are merely laboratory notes, no attempt has been made to furnish references to the sources from which the procedures have been obtained, and full acknowledgment is hereby made to the numerous authors on whose contributions the following descriptions are founded. Many of the tests as given are modifications developed in the laboratory from the original procedures, and are here published for the first time. But it is not presumed that these are the only methods which might or should be employed. The methods given here represent merely the practice at St. Luke's laboratory, and are such as we have found satisfactory after long use. The somewhat peremptory and dogmatic style in which the directions are written is explained by the fact that they have been prepared for the use of internes in the pathological laboratory, as stated above, and therefore, have been compressed as much as possible, all irrelevant matter being omitted.

The writer is responsible for the chapter on histological methods, and, in conjunction with his colleague, Dr. Karl M. Vogel, has revised the notes on clinical pathology. Dr. Vogel bears the entire responsibility for the chapter on clinical analytical methods; and Dr. L. W. Famulener has prepared the sections on bacteriology and serology.

F. C. WOOD.

HISTOLOGY

PREPARATION OF HISTOLOGICAL SPECIMENS

GENERAL DIRECTIONS.

Specimens from the operating room are put into jars of 4 per cent. formalin, or, if large, are wrapped in moist towels or gauze, and placed in the ice-box. The date, the patient's name, (surname first), the ward, the name of the operator, and the nature of the specimen are entered in the day-book. (The assistant attending surgeon's name is used only when cases are sent special to him. The house surgeon's name is never used). The name, the ward, the date, and the name of the operator are entered on description card, name card, and diagnosis card (Fig. 1). The day-book number is placed on the jar, and on the three cards, A brief macroscopical description is written on description card (Fig. 1, A), and relations, dimensions of tumors, etc., are given.

One or more pieces for section are cut from the specimen and placed in small bottles of 5 per cent. formalin with the number of specimen on the label. This should be done immediately on the receipt of specimens too large to preserve whole; such large specimens should be submitted to the pathologist to determine whether they are to be preserved in Kaiserling or not. The pieces removed should include the characteristic portions, and if the lesion involves the skin or mucous membrane, the plane of incision should be at right angles to the surface. In all tumors, care should be taken to obtain portions of the adjacent lymph nodes if they are present. When the uterus and adnexa are sent down, there should be cut at least one block from the uterus showing endometrium, one from each tube, and one from each ovary. Unless otherwise requested by the surgeon, all large specimens should be divided like a book with parallel cuts about one inch apart, running nearly through the specimen for distinctive portions, and for proper penetration by the fixative. The

3341 Carcinoma of breast and axillary lymph nodes
NAME Smith Mary Ward M2
Operator Dr Jones May 6, 1916
Macroscopical Examination Specimen consists of breast and axillary contents. Breast measures 16 x 12 x 6 cm. In the upper and outer quadrant is a small tumor, which on section is very firm, and yellowish in color. Tumor is roughly spherical and measures 2 x 2 x 3 cm. The nipple is not retracted and tumor not adherent to skin. The axillary fat contains lymph nodes, the largest 1 cm in diameter
Microscopical Examination Tumor is composed of alveoli, solidly filled with cells having large clear nuclei, scattered
B
3341 Carcinoma of breast and axillary lymph nodes
NAME Smith Mary Ward M2
Operator Dr Jones May 1 1916
C
3341 Smith Mary Ward M2
Operator Dr Jones May 6 1916
Diagnosis.
Carcinoma of Breast and axillary lymph nodes
F. E. Wood
A

Fig. 1. Cards used in recording surgical material (about half size). A, Description card, filed according to serial number. B, Diagnosis card, filed according to anatomical region. C, Name card (blue) filed alphabetically.

blocks are best cut roughly for formalin, and, after they have been in the 95 per cent. alcohol, trimmed to pieces 3 to 5 mm. thick, and not much over 2 cm. in diameter.

Tissue blocks are then embedded as follows, unless they contain bone or calcified areas. For large tissue blocks for which there is no hurry in making a report:

1. 4 per cent. formaldehyde, 12 to 24 hours;
2. 80 per cent. alcohol, 2 hours;
3. 95 per cent. alcohol, 2 hours;
4. 95 per cent. alcohol, overnight;
5. Absolute alcohol, two changes in 24 hours. The number of changes will depend upon the bulk of the blocks in the bottle. If only one or two small blocks are to be dehydrated, two changes of a small quantity of alcohol will suffice. If the bottle is half full of tissue, it will be best to make three changes;
6. Equal parts of carbon disulphide and absolute alcohol. The blocks should sink in this mixture and become transparent. When this occurs, they are sufficiently penetrated. For a thin specimen only 1 hour may be required; for thicker specimens 3 to 4 hours. If the blocks do not clear up, they still contain water and must be returned to the absolute alcohol for further dehydration;
7. Pure carbon disulphide, 2 to 6 hours. The blocks float in this at first and become somewhat cloudy. Usually they sink to the bottom after some hours, but occasionally not at all. If the specimen contains a large amount of fat, two changes of carbon disulphide should be used. The alcohol being removed by the carbon disulphide, the preparation is transferred to
8. Carbon disulphide saturated with paraffin at room temperature. Such a solution contains 16 to 20 per cent. of paraffin. In this the blocks sink and again become quite transparent, and the material can remain in such a solution for months without deterioration. This step can be omitted if haste is necessary, and the blocks transferred at once to
9. Carbon disulphide saturated with paraffin at incubator temperature, 37 to 40 C. This contains 68 gm. of paraffin to 100 c.c. Moderate sized blocks should be left in this 37 C. carbon disulphide and paraffin for 1 to 2 hours if the tissue is very thin,

or for several days if more than 1 cm. thick. Prolonged treatment at this temperature does not make the tissues hard or brittle. When the blocks are perfectly clear, which usually occurs in four to six hours in routine specimens, they are transferred to

10. Stock melted and *filtered* paraffin at 55 C. for 1 hour, then to a fresh lot of paraffin, not previously used, for 1 hour.

11. A shallow tin dish is filled with the freshly melted paraffin not previously used for embedding purposes, a strip of paper is laid on the bottom, carrying the number of the specimen in pencil, and the pieces of tissue are arranged in the dish with a hot needle. It is convenient to place on the bottom of the dish the surface from which the section is to be cut. Bubbles can be removed with hot needle. If a skin forms over the surface, it can be removed by a moment's flaming with a Bunsen flame. When the blocks are all arranged satisfactorily in the dish, it is floated immediately on cold water so as to cool the paraffin as rapidly as possible. If the inside of the dish has previously been rubbed with a trace of glycerin, the paraffin will separate from the walls easily. If not, the blocks must be cut out with a knife. The dishes are conveniently kept in the ice-box, as the contraction may cause the paraffin to crack away from the walls and so free the block without cutting.

Care must be taken that the specimen is never left in the paraffin at 55 C. for more than two hours, as the tissues are very much injured by heat. If the specimen contains any water, the heat of the paraffin bath will ruin it.

In cutting out such embedded blocks, a thin-bladed knife should be used, and the material should be sawed rather than cut, because if direct pressure is exerted the block may crack in a number of directions, and the cracks pass through the tissue. Each piece of tissue is cut from the cake and trimmed carefully, always leaving a wall of paraffin, the walls of the block being cut perpendicularly to the surface and at right angles to each other. The trimmed tissue and paraffin are fastened to a fiber block after the surface of the latter has been heated in a Bunsen burner or over an electric stove, the thickest side being placed against the hot block, where it will adhere owing to the melting of the paraffin. Care should be taken that the tissue itself is not heated by the hot fiber, as this will interfere with the staining.

The fiber block is immediately numbered with a lead pencil. After it has cooled, the surplus paraffin is trimmed close to the sides of the tissue, leaving a little more at the ends to permit of satisfactory ribboning of the specimen. The blocks can be kept in the ice-box until they are to be cut, when they are clamped in the microtome with the long diameter of the block perpendicular to the edge of the knife.

The microtome knife should be stropped and placed at an angle of about 15 C. to surface of block, and adjusted so that it just clears the block. The feed is adjusted to give four clicks (about 13 micra in the International Instrument Company's Minot model microtome), and thick sections are cut until the whole piece of tissue appears in sections. The feed is then adjusted to two clicks (about 7 micra); a new portion of the knife is selected; and ribbons of sections are spread on a pan of distilled water at about 48 C. on electric stove. Even, flat sections are fished to two or three clean slides, and the slides are numbered with a diamond pencil and stood on end to drain. If desired, in order to prevent the sections from coming off the slides, the slides may be rubbed over with a very small amount of egg albumin solution with the finger before mounting the sections. They are then placed in trays in the oven at 37 C. for twelve hours to dry (unless "rush"; see special technique). The slides should be covered by a sheet of paper or cardboard to prevent dust settling on the preparations. When dry the paraffin should be melted by placing the tray in the oven at 55 C. for a few minutes. After cooling the slides are transferred to a jar of xylol.

Sections are prepared and stained as follows:

1. Xylol, about 2 minutes;
2. Xylol, about 2 minutes;
3. Alcohol absolute, 2 minutes;
4. Alcohol absolute, 2 minutes;
5. Alcohol, 80 per cent., 2 minutes;
6. Water, 2 minutes;
7. Hematoxylin, about 5 minutes (control with microscope);
8. Distilled water, 10 minutes;
9. Tap water, 10 minutes (better 1 hour):
10. Alcohol, 80 per cent., 2 minutes;

11. Eosin, ½ to 2 minutes;
12. Alcohol absolute, 2 to 3 minutes;
13. Alcohol absolute, 2 to 3 minutes;
14. Alcohol absolute, 2 to 3 minutes;
15. Carbol-xylol, 1 minute;
16. Xylol, 1 minute;
17. Mount in gum dammar; label with number.

The diagnosis is written on the blue name card (Fig. 1, C) by the pathologist, and signed; it is then copied exactly by the interne on the description card (Fig. 1, A) and the white diagnosis card (Fig. 1, B), and in the day-book. A duplicate of the diagnosis card is filled out and sent to the attending surgeon.

A brief microscopical description is written by the interne on the description card, and handed to the stenographer to be copied. When returned, the card is filed by number. The small white card is filed according to anatomical region, the blue card by name.

If a previous specimen from the same patient has been examined, the number of the former entry should be noted on the blue card and in the day-book before the section is presented for diagnosis. One slide of each representative block (or more if of special interest), is filed in the slide cabinet by number.

Gross specimens are invariably kept until after the diagnosis is signed. Specimens too large for preserve jars are tied in gauze with a permanent tag bearing their numbers in lead pencil, and placed in crocks or anatomical jars. A special crock is kept for fibroids, and another for breast tumors. When the attending pathologist signs the diagnosis, he should be asked what disposition is to be made of the specimen. If it is thrown out, the day-book entry should be stamped "Spec. not saved." The blocks also are kept until the diagnosis is signed. If saved, they are labeled with a paster bearing number, and filed in numbered pasteboard boxes.

The interne histologist is responsible for:

Care of surgical specimens, and cutting and staining of sections.

All records of such specimens and the report to the operator.

Care of microtome, paraffin, knives, and stains.

No diagnosis is to be reported unless signed by the attending or resident pathologist.

ZENKER'S FIXATIVE.

When Zenker's solution is used for fixation, the changes in the technique are as follows: Specimens not to be over 5 mm. in thickness.

Place in Zenker's solution for 6 hours.

Wash in running water for 24 hours in a Fairchild porcelain cup or a wide-mouthed bottle covered with gauze, through which a glass tube drawn to a point is thrust. The tube is connected with the water supply and a slow stream is allowed to flow in. Proceed as after formalin above.

In staining: After the 95 per cent. alcohol wash for 10 to 20 minutes in tincture of iodine to remove the mercuric chloride, and then wash, till all brown color is removed, in 95 and 80 per cent. alcohol. A treatment with a 5 per cent. aqueous solution of sodium hyposulphite (fixing hypo) will remove the iodine very completely. Wash in distilled water, then follow regular routine. It is often difficult to stain Zenker's preparations with hematoxylin; if trouble is experienced, place the section over night in a very dilute (1:50) Delafield's hematoxylin and decolorize with 1 per cent. hydrochloric acid alcohol if necessary. Or, if preferred, Weigert's iron hematoxylin can be used for the usual length of time.

CARE OF KNIVES.

Honing.

Microtome knives are honed on a yellow Belgian water hone, used for them only. Wet the surface with water or water and glycerin in equal parts. Hone against the edge from heel to toe, always making the full stroke. The knives are hollow ground, so keep the back in contact with the stone and do not press the blade against the stone, merely steady it with the fingers of the left hand. Hone until the edge, or a usable portion of it, is free from nicks when examined under the microscope with a low power. If the knife has been injured by contact with bone, hone on a so-called carborundum razor hone, using either soap and water or glycerine and water until the nicks are removed, then finish on the Belgian hone. Hone the knife about once a week.

Stropping.

Strop away from edge from toe to heel, also keeping back

edge in contact with strop. Strop until edge appears as straight line with polished borders, and no serrations. Strop between every ten blocks.

Wipe knife with xylol and put in case after using. A small amount of diamantine powder No. 2 may be rubbed into the strop occasionally to increase the sharpening power. When the knife is in proper condition it should easily cut a loosely held hair.

SOLUTIONS.

The staining and clearing solutions are kept in a series of Hellendahl or Coplin staining pots.

Ninety-five per cent. alcohol: kept in three Hellendahl jars. Jar No. 1 is emptied daily into waste bottle, for redistillation of alcohol. No. 2 is emptied into No. 1, and No. 3 into No. 2; and No. 3 is then filled with fresh alcohol.

Absolute alcohol: kept in original bottle or in Coplin jars. Staining jars before carbol-xylol are refilled every week; after second xylol, every two weeks.

Eighty per cent. alcohol: made by diluting 95 per cent., 84 c.c. to every 100 c.c.

Carbon bisulphide *technical.*

Carbon bisulphide and absolute alcohol: made by mixing equal parts.

Carbon bisulphide and paraffin: made by saturating carbon bisulphide with paraffin at 37 C.

Carbon bisulphide is very inflammable. Do not put it in the oven, or carry a lighted match near the bottle. All bottles containing this substance are to be kept *loosely* corked so that any vapor which may be generated from the heat of the room will not blow out the cork.

Carbol-xylol: Stock made by mixing carbolic acid crystals one part and xylol three parts by volume. In order to measure the carbolic acid, it may be melted by standing the bottle in hot water. It should be removed in three days to one week.

Dammar: made by dissolving gum dammar in xylol and neutralizing by shaking up the solution with precipitated chalk. The xylol is frequently acid in reaction.

Eosin stock solution: made by saturating 95 per cent. alcohol with precipitated eosin. The latter is obtained by precipitating

Grübler's "Eosin wasserlöslich gelblich" from a strong aqueous solution with hydrochloric acid; and then filtering and washing the precipitate until it is acid free. The contents of the filter are allowed to dry at room temperature, powdered, and preserved in a stoppered bottle. For use, the stock solution is diluted with 95 per cent. alcohol about 1:10. The stain will keep indefinitely and the jar may be filled up as the solution evaporates.

Hematoxylin: made by diluting stock Delafield hematoxylin 1:10 with water. The solution keeps for a week or two, but must be filtered daily.

Ammonia water: one drop of strong ammonia in jar of distilled water.

Thin celloidin: a 4 per cent. stock solution diluted 1:10 with equal parts of 95 per cent. alcohol and ether.

SPECIAL TECHNIQUE.

FROZEN SECTIONS.

When a frozen section is ordered for an operation, notify the attending or resident pathologist at once. Have ready a tank with plenty of carbon dioxide, a sharp chisel knife, a tray of water, needles, smooth pointed forceps, a camel's hair brush, four clean slides and covers, two glass "tooth powder dishes", a bottle of saturated aqueous solution of thionin, and smooth surfaced blotting paper.

Place the specimen on a freezing microtome attached to the carbon dioxide cylinder; allow the gas to flow for a few seconds; then turn it off for half a minute, and repeat this process, twice if necessary. Take care to avoid freezing the specimen too hard, as the knife cannot cut through the tissues when they are over-frozen. If over-freezing does occur, allow the upper surface of the specimen to thaw until the proper consistency for cutting is reached. Hold the knife at an angle of 30 to 40 degrees above the horizontal, and cut a number of sections rapidly, shaking them from the blade into water. With fine-pointed forceps, seize a suitable section and dip it, for two seconds, into a dish containing 0.5 per cent. aqueous thionin solution; then still holding it with the forceps, remove the section to a dish of water and wash it free of excess of dye. Then slip a slide under the section in the water and gradually raise it, so as to leave the tissue floating

in a little pool on the slide. When the section is perfectly flat, remove the water by applying the edge of a strip of blotting paper to the border of the drop, not touching the tissue itself. Place a cover-glass over the preparation, and examine it with a microscope. The nuclei are stained blue, the connective tissue pinkish, the mucous tissue pink, and colloid blue.

The section can be preserved for a few days by mounting it in Bruns' glucose medium, which is made by dissolving 40 gm. glucose and 10 c.c. glycerin in 140 c.c. distilled water.

"Rush" specimens for permanent mounts may also be cut frozen as follows: After hardening overnight in formalin, cut block not over 1.5 cm. square, wash, place on freezing microtome table, freeze, and cut. Fish thin sections on to slide under water with needle, drain, flood with absolute alcohol, blot hard, and cover with thin celloidin. Then soak the sections in water, stain with hematoxylin, and dehydrate as usual, but use only 95 per cent. alcohol, not absolute alcohol.

When it is desired to block rush specimens in paraffin, the regular routine may be shortened as follows. Very thin small blocks must be used.

		Routine	Rush
1	4 per cent. formaldehyde	12 hrs.	15 min.
2	80 per cent. alcohol	2 "	
3	95 per. cent. alcohol	2 "	15 "
4	95 per cent. alcohol	12 "	
5	1st absolute alcohol	12 "	15 "
6	2nd absolute alcohol	12 "	30 "
7	Carbon bisulphide and alcohol (until block sinks)	4 "	10 "
8	Carbon bisulphide (or until clear)	6 "	30 "
9	Carbon bisulphide and paraffin (cold)	4 "	
10	Carbon bisulphide and paraffin (hot)	12 "	10 "
11	1st paraffin	1 "	30 "
12	2nd paraffin	1 "	30 "
13	3rd paraffin	embed	embed

BONE SECTIONS.

Tissue containing bone or calcareous masses, after thorough fixation in formaldehyde or alcohol, is washed for a short time in water, then placed in a solution of strong nitric acid (7.5 c.c. of acid in 92.5 c.c. of water); which is changed every few hours until no bone can be detected on piercing the tissue with a fine needle. The tissue is then washed for 24 hours in running water, using a 2-way cork in bottle, and embedded in the ordinary way, beginning again with 80 per cent. alcohol. If the staining qualities of the tissue are poor, it may be soaked in dilute ammonia water for ten minutes. Sections are cut with a microtome knife with a heavy blade, especially kept for this purpose so as to avoid injuring the one used for regular work.

STAINS.

DELAFIELD'S HEMATOXYLIN.

The solution is made as follows:

100 c.c. of saturated solution of ammonium alum.*

4 gm. of hematoxylin dissolved in 6 c.c. of 95 per cent. alcohol.

The mixture forms a purple solution which must be exposed to light and air for about a week, until it becomes somewhat more reddish. Then 25 c.c. each of glycerin and commercial methyl alcohol are added as preservatives. The solution is filtered and stored in stoppered bottles. The stain keeps for about two years in well filled, tightly stoppered bottles, ripening slowly and becoming more powerful and also more acid from decomposition of the alum. If the solution must be ripened quickly it may be exposed in a layer 2 cm. thick in a dish covered with filtered paper for three days. When diluted 1:10, this solution will stain nuclei is about fifteen minutes, whereas the same solution kept in a loosely stoppered bottle at the end of three months will stain nuclei the same depth of color in from two to three minutes.

Delafield's hematoxylin should never be used in concentrated form, but should be filtered and diluted from five to twenty times with *distilled* water. The solution so diluted does not keep for

*About 11 per cent. of the crystallized salt.

more than ten days, and an iridescent scum is apt to form on the surface. This scum should be removed by filtration as soon as it forms, for it may adhere to the surface of the sections as a blue or black deposit, and ruin them.

Sections must always be rinsed in *distilled* water after staining, as tap water usually causes a precipitate of black particles on the surface of the sections. After such rinsing, the sections are improved by a thorough washing in tap water, which by virtue of its slight alkalinity removes any acid from the tissues, and alters the color of the nuclei from a violet to a deep blue.

A solution which requires no ripening may be obtained by using hematin (Grübler) in place of hematoxylin, though even this solution does not reach its full strength for some days.

The hematoxylin may also be oxidized into hematin, as suggested by Harris, by adding 0.5 gm. of mercuric oxide to each 100 c.c. of the freshly made alum-hematoxylin solution and boiling. As soon as the liquid becomes a dark reddish purple color it is removed from the flame and cooled rapidly. The glycerin and methyl alcohol are then added. The stain may be filtered and used immediately.

If a pure nuclear stain is desired, the addition of 2 c.c. of glacial acetic acid to 100 c.c. of the Delafield hematoxylin will give an acid hematoxylin which practically does not stain the cell body. Or, if preferred, the sections may be left in Delafield's hematoxylin until they are slightly overstained, and then the excess of dye removed by decolorizing with acid alcohol containing 1 per cent. of hydrochloric acid in 95 per cent. alcohol.

WEIGERT'S STAIN FOR ELASTIC TISSUE.

1 Lithium carmin, 10 to 20 minutes;
2 Acid alcohol, 2 to 10 minutes;
3 Weigert's elastic tissue stain, 25 to 30 minutes;
4 Alcohol;
5 Absolute alcohol, 2 to 18 hours, to differentiate;
6 Xylol; balsam (do not use carbol-xylol).

Preparation of Stain

Basic fuchsin	2 gm.
Resorcin	4 gm.
Water	200 c.c.

Heat mixture in porcelain dish; and when boiling add 25 c.c. of liquor ferri sesquichloridi. Filter the solution and allow the precipitate to drain until nearly dry. Return filter paper to same dish, and cover with 200 c.c. of 95 per cent. alcohol; boil; filter; make up to 200 c.c. with 95 per cent. alcohol; and add 4 c.c. of concentrated hydrochloric acid.

WEIGERT'S IRON HEMATOXYLIN STAIN.

After sections have gone through the xylol, alcohol and distilled water, mix equal parts of Weigert's No. 1 and No. 2, and stain for 5 to 10 minutes. Wash thoroughly in several changes of water for half an hour, then put through eosin, the alcohols, and xylols, and mount in gum dammar. In the original method a picro-acid fuchsin counterstain was used, but this fades rapidly. This is the best stain for old material which has been kept for a long time in alcohol. Sometimes decalcified bone will stain by this method.

FAT STAINS.

The most satisfactory method to demonstrate fat is that employing some of the anilin dyes. Of these Sudan III and Scharlach R are the most suitable. The tissues are fixed in 4 per cent. formaldehyde or formalin (Müller's solution), and, after cutting on a freezing microtome, the sections are transferred to 50 per cent. alcohol, and then to the staining solution. This consists of a saturated solution of either of the above mentioned dyes in a mixture of equal volumes of acetone and 70 per cent. alcohol. Usually ten or fifteen minutes are sufficient for staining. Sections are then rinsed in alcohol of 60 to 70 per cent. strength and transferred to distilled water. If desired, nuclei can be lightly tinged with dilute Delafield's hematoxylin. The sections may be mounted in glycerin jelly or levulose syrup.

Fat particles are colored orange by Sudan III and red by Scharlach R. Fatty acid crystals do not stain. Myelin, lipochrome, lecithin, and "protagon" droplets are also stained.

MARESCH-BIELCHOWSKY STAIN FOR CONNECTIVE TISSUE FIBERS.

Tissues are fixed in formaldehyde and embedded in paraffin. After cutting the sections are treated as follows:

1 Placed in 2 per cent. silver nitrate solution, 12 to 24 hours;

2 Washed quickly in distilled water, 5 seconds;
3 Stained in ammoniacal silver solution, 2 to 30 minutes; according to thickness of section;
The solution must be prepared fresh, as follows: To 20 c.c. of 2 per cent. silver nitrate solution add 3 drops of 40 per cent. sodium hydrate solution, and then add ammonia drop by drop until the precipitate is dissolved.
4 Washed in distilled water;
5 Reduced in 8 per cent. formaldehyde until section is colored gray or gray brown; (Reduction takes place place almost instantly.)
6 Placed in gold bath, 10 minutes;

1 per cent. aqueous solution of gold chloride,	3 drops
Distilled water,	10 c.c.
Glacial acetic acid	3 drops

7 Placed in 5 per cent. solution of hyposulphite of soda, 15 to 30 seconds;
8 Washed in distilled water;
9 Fixed in slide with albumin;
10 Dehydrated in alcohol;
11 Cleared in xylol;
12 Mounted in dammar.

STAIN FOR TUBERCLE BACILLI.

1 Delafield's hematoxylin, 20 to 30 minutes;
2 Water, 30 minutes;
3 Carbol fuchsin, 1 hour at 37 C. (incubator);
(Place slide in Petri dish and flood it; then cover to prevent evaporation.)
4 Decolorize in acid alcohol, 1 minute;

Hydrochloric acid	1 c.c.
95% alcohol	70 c.c.
Water	30 c.c.

5 Wash in 70 per cent. alcohol, 2 to 3 minutes;
6 Wash in water;
7 Lithium carbonate solution until section takes blue color;

Sat. sol. lithium carbonate	1 part
Water	10 parts

8 Wash in water, 5 minutes;
9 95 per cent. alcohol, 5 minutes;
10 Absolute alcohol, 5 minutes;
11 Xylol, 5 minutes;
12 Mount in gum dammar.

To obtain the best results the material should be fixed in 95 per cent. alcohol, though formalin preparations occasionally stain well. After fixation in Zenker's solution, the bacteria do not stain satisfactorily.

CLINICAL PATHOLOGY

BLOOD.

A "leucocyte count" includes an estimation of the number of leucocytes, based on a count of 400 small squares at a 1:20 dilution, multiplying factor 200. If the number of leucocytes is above 50,000, a 1:100 dilution may be used or only 100 squares counted. A differential count of 200 cells is made on a slide stained with Jenner. If the count is 9,000 or below, 100 cells are sufficient for a differential except in cases of special interest. In making red or white cell counts or differential counts the use of an automatic tally register (obtainable under this name in sporting goods stores) is of service. In making a differential count, record on the instrument each leucocyte seen, and note, either mentally or on paper, all varieties except polynuclears. When the total reaches 100 or 200, as the case may be, the number of polynuclears is obtained by subtracting the sum of all the other varieties from the total number.

In routine differentials the cells are classified as follows:

Polymorphonuclears: Cells with irregular nuclei and neutrophile granules and forms with kidney-shaped nuclei and neutrophile granules.

Lymphocytes: All mononuclear cells not distinctly granular.

Eosinophiles: Polymorphonuclear cells with eosinophilic granules.

Basophiles: Polymorphonuclear cells with basophilic granules.

Large mononuclears: Cells with large, pale, oval nuclei and no granules with the Jenner stain. If abundant they should be separately enumerated. If not, they may be included with the lymphocytes.* Transitionals resemble the foregoing but have

* Strictly speaking, the large mononuclears and transitionals belong with the myeloid group of cells, since they give the oxidase reaction.

kidney-shaped or somewhat irregular nuclei. In counting they are grouped with the large mononuclears.

Myelocytes: Cells with large, pale, round or oval nuclei and neutrophilic, eosinophilic, or basophilic granular protoplasm.

The usual form of record is:

"W. B. C.: 12,500, Polys: 78, Lymph.: 21.
Eos.:1/2, Bas.: 1/2"

In cases in which the blood picture is of special interest, as in leukemias, severe anemias, malaria, etc., the large mononuclears and transitionals should be noted and charted in separate groups.

A "red count" includes an estimation of the number of cells per cubic mm. based on a count of at least 100 small squares at a 1:200 dilution for approximately normal blood or 1:100 for anemic blood, and a description of the morphology of the cells based on examination of a smear stained with Jenner. Note inequality in size (anisocytosis), irregularity in shape (poikilocytosis), or any increase in central depression. Look for abnormally large cells (macrocytes), or abnormally small cells, (microcytes), for cells that stain diffusely blue, i. e., polychromatophilia, or show basophilic granulations, or larger basophilic particles (Howell-Jolly bodies).

In anemias, look for nucleated forms which are classified as normoblasts (cells 10 micra in diameter or less), and megaloblasts (cells over 10 micra in diameter). Large and atypical forms are often found along the borders of the smear and at the end where the last cells are deposited.

A "hemoglobin determination" consists in an estimation made with the Oliver or Fleischl-Miescher instrument.

A "malaria hunt" consists in a careful search of thin smears stained by Wood's stain, using an oil immersion lens and a mechanical stage, and may be considered as negative if no parasites are found after fifteen minutes. If quinine has been given, a longer search may be necessary or thick decolorized smears may be studied. (See Wood: Chemical and Microscopical Diagnosis, 3rd ed., 1912, p. 87).

TECHNIQUE.

Leucocyte count: (See Wood: Chemical and Microscopical Diagnosis, p. 45). Blood is obtained from finger tip or ear by punctur-

ing with a Hagedorn needle. When not in use the needle is kept with point immersed in a bottle of 95 per cent. alcohol. The skin of the patient is cleansed with alcohol before making the puncture. The puncture must be large enough so that slight pressure at a distance of one-half inch causes a large drop to appear. The first drop is wiped away, and the second used.

Blood is drawn in a 1:10 pipette, exactly to the 0.5 mark. The pipette is held horizontally and at right angles to the line of vision. For accurate work the blood cannot be drawn beyond the mark and then blown out. Still holding the pipette in a nearly horizontal position, the tip is wiped with the finger and dipped into a bottle of 2 per cent. acetic acid held tilted on its side. The acetic acid must be filtered every few days. The acid is drawn up until the mixture reaches the 11 mark, rotating the pipette slightly. As soon as the bulb begins to fill, the pipette is changed to a vertical position to avoid including a bubble. When filled, the pipette is gently shaken for half a minute, with a finger held firmly over the end. If a second drop is taken from the pipette it must be again shaken for half a minute. Two or three drops are then allowed to flow out so as to empty the stem, and a small drop is placed in the center of the counting table. Time may be saved by making two chamber preparations, then if one is not satisfactory, the other may be. The drop should be just large enough to cover the table when flattened with a cover slip. It must on no account be large enough to spread beyond the gutter and wet the rim on which the cover rests, or so small as not to cover the rulings. If the cover glass and counting chamber are clean and in optical contact, Newton's rings will be visible when the chamber is held at the level of the eyes. In handling the filled chamber be careful always to keep it horizontal. The presence of an air bubble in the preparation makes it unsatisfactory for counting. A moment is allowed for the cells to settle and the number on one square millimeter (one of the nine large squares into which the Zappert-Ewing field is divided) is counted, using a number 3 objective and a number 3 eye-piece. Cells touching the left or upper boundary line are considered in the square, those touching the right or lower boundary as out of the square. Count at least four square millimeters, preferably in two different preparations, divide the total number of cells by the number of square mm. counted and multiply by 200 to obtain the number

per cubic mm. of undiluted blood. The number of cells in each square should not vary more than 25 per cent. of the average number per square. The value of the count depends chiefly on the accuracy with which the blood is drawn up, and the thoroughness of the mixing. Chart result as the nearest multiple of 500.

Red Cell Counts: (Wood, p. 39). These are made similarly, using a 1:100 pipette, drawing the blood up to the 0.5 mark, or to 1.0 if patient is very anemic, and diluting to the 101 mark with Hayem's solution or 3 per cent. sodium chloride solution. The chamber is filled under the same precautions as in making the white counts, and 100 small squares counted in four blocks of 25 each. The sum of the cells counted is multiplied by 8,000 to obtain the number of cells per cubic mm. The counts of the different blocks should be within 20 per cent. of each other. A very common source of error is delay in putting the cover glass in position, resulting in counts that are too high. Chart result as the nearest multiple of 100,000. If Bürker's chamber is used the calculation is made as follows: For red cells at a dilution of 1:200 count all the cells in eighty of the smallest squares (each equivalent to 1/400 square mm.), point off two places, and the result will be the number of cells expressed in millions. For white cells at a dilution of 1:10 count one hundred of the larger squares, (each equivalent to 1/25 square mm.), and multiply by 0.025 to obtain the number of cells in thousands.

Counting Blood Platelets. A 1:100 dilution is made in a red cell pipette, from the second drop of blood emerging from a fresh puncture, the diluting fluid being freshly mixed as described in the section headed "Formulæ". Fill the counting chamber as usual but use a special thin cover so that the counting may be done with a high power dry objective. Allow the preparation to stand about ten minutes before counting so that the platelets will all have settled to the bottom of the cell. The red cells will appear as indistinct shadows, the leucocytes will show deep blue nuclei, and the platelets will appear as round or oval lilac colored bodies. Normally they number about 300,000 per cubic millimeter.

Oliver Hemoglobinometer. (One set of discs for this instrument is marked "Day light" and the other "Candle light", and only the corresponding type of illumination must be used.)

A large drop of blood is obtained, using special care not to squeeze out tissue fluids, and the tip of the small capillary tube

is touched to the drop. If the tube is clean and is held horizontally, it will fill promptly. The blood is then quickly wiped from the outside of the tip with finger, using care not to draw any blood out of the capillary, and leaving the column flush with the ends of the tube. The pipette is then filled with distilled water up to the 700 mm. mark (accurately) and the water blown through the capillary tube into the cell with the porcelain bottom so as to wash all the blood into the cell. It is mixed by stirring with the capillary tube and covered with the pale blue glass cover —forcing a drop into the metal chamber. The cell is then placed beside the scale so that the two are evenly lighted, and observed through the "telescope" holding one hole over the cell and the other over a disc on the scale. The scale is moved backward and forward until the disc which most nearly matches the diluted blood is obtained. The reading is made directly from the number above the scale disc. If the color is well between that of two of the discs, say 60 and 70, the percentage is considered as 65. Five per cent. is as close as one can expect to read.

Pink riders to be placed over the standard discs are provided for greater accuracy, but are of doubtful value. The darker rider, marked "5" adds 5 per cent. to the reading when placed over one of the standard discs of the upper half of the scale. The paler, marked "2.5" adds 2.5 per cent. to the upper six discs, and 5 per cent. to the lower discs. The capillary is cleaned by passing through it a needle threaded with soft cotton. The cell should be dried after using.

Fleischl-Miescher Hemoglobinometer (Wood, p. 26.) Blood is taken in the 1:200 mixing pipette to the 1.0 mark, and 0.1 per cent. sodium carbonate or distilled water is drawn in to the mark above the bulb. After slight shaking the pipette may be laid aside, but only for a short time, as the color of the solution rapidly changes. The 15 mm. cell is cleaned and dried, and the bottom gently screwed on. One side of the chamber is filled with distilled water. The first drops of the fluid are blown from the pipette, and the other side of the cell is then filled with the laked blood until a convex meniscus projects. The grooved cover slip is slid on so as not to include air bubbles and not to force the solution over the partition. The metal cap is put on with the slit at right angles to the partition. The cell is placed in the stage so that the "water" half lies over the glass wedge. The candle is

lit and the light adjusted; the dark box is covered and the reading is made through the tube. The wedge is slid forward and backward so as to make the near half of the field first too dark and then too light, until as nearly perfect a match as possible is obtained. Two independent readings should be made and averaged, and the nearest multiple of 5 used as the result per cent.

The accuracy of the readings can be greatly increased by proper use of the pipette. If the blood is drawn one sub-division beyond or short of the 1.0 mark, 1 per cent. is subtracted or added to the reading, as the case may be. Percentages below 30 are best determined by making a 1:100 dilution in an ordinary large bulb red blood counting pipette, and dividing the result by two. These irregular dilutions should be noted in writing when the blood is taken, to avoid confusion.

Cleaning Pipettes. Pipettes are cleaned by drawing through them, first, distilled water, then alcohol, then ether, then air. The bead should be freely movable without sticking to the side of the bulb, and the bulb should be dry and clean. If blood has dried in the pipette it should be removed with a horse hair or fine brass wire, such as is used for hypodermic needles, and the pipette then cleaned by filling with nitric acid or strong antiformin and allowing it to stand so over night. The Fleischl-Miescher cell should be taken apart, cleaned, and dried after use.

Blood smears for differential count or morphological examination are made on new slides. A differential count should always be made with a leucocyte count and a morphological examination with a red count. The slides are moistened with the breath and vigorously polished with a cloth. This gives them a slight warmth which is of advantage. A drop of blood is taken on the end of another slide as soon as it appears at the puncture, and is smeared by dragging, (or pushing), it across the first slide. The smearing slide is held at an angle of 45 degrees and should touch the first slide lightly. The smear is dried as *quickly as possible* by waving it in the air, or blowing on it and at the same time rubbing the back to warm it slightly. Smears for morphological examination or malaria hunts must be so thin that the red cells lie flat without touching each other. For differential counts they may be thicker, provided the leucocytes are flattened out enough to show their morphology clearly.

Fresh preparations of blood are made by taking a very small

drop of blood on the center of a cover slip, which has been cleaned very carefully in soap, water, and alcohol, and laying it on a slide similarly cleaned. If the glass is clean and the drop small, the blood will spread out, leaving the red cells separate. If the preparation is to be kept under observation for some time seal the edges of the cover glass to the slide with a little vaseline or cedar oil.

Jenner Stain. This is used for practically all purposes except malaria hunts. The slides are stood in a Coplin jar filled so as nearly to cover them with Jenner stain, and left three to five minutes. They are then removed, rinsed in one jar of distilled water, and washed in another, until of a lavender color, then blotted dry. The longer they are washed the more blue is removed and the pinker they appear. *Keep the Jenner jars covered.*

Wood Stain. Smears are fixed for three to five minutes in methyl alcohol, and blotted dry. They are then covered with 0.1 per cent. eosin for one minute; the excess of the stain is poured off, and a few drops of 0.25 per cent. methylene azure I are added for thirty to forty seconds. Longer treatment gives deeper stain. They are washed in distilled water and blotted dry. The stain can be made as desired by adjusting the length of time that each solution is allowed to act (Wood, p. 87).

Oxidase Reaction.

Fix the films by exposing to the vapor of strong formaldehyde under a bell jar for ten to fifteen minutes, or preferably use 4 per cent. formaldehyde for two to three hours. Dissolve a knife point of para-amido-dimethyl-aniline-sulphate in 2 c.c. of 1/10 normal NaOH solution and add an equal volume of 1 per cent. aqueous solution of alphanaphthol. Flood the specimen with this, apply a cover glass, and examine with the high power dry lens. All myeloid cells, that is, polynuclears, myelocytes, large mononuclears, transitionals, and myeloblasts will show blue granules, while lymphocytes and lymphoblasts will not. Compare a preparation made in this way with one stained with Jenner to form an idea of the relative proportion of cells giving and not giving the oxidase reaction.

Determining the Resistance of the Red Blood Cells.

This may be done on (a) unwashed, or (b) washed, cells, the former method being somewhat simpler. A series of accurately graduated salt solutions is prepared and kept ready for use. Weigh on a chemical balance 5 gm. of c. p. NaCl that has been dried in the oven at 120 C. for an hour or more, and make up to 500 c.c. with distilled water in a volumetric flask. From this 1 per cent. solution prepare a series of dilutions so that strengths ranging from 0.3 per cent. to 0.6 per cent. are obtained, varying by intervals of 0.025 per cent. Keep in *tightly stoppered* bottles.

(A). Unwashed cells. With a red cell counting pipette having a large bulb or Fleischl-Miescher hemoglobin pipette draw up blood to the 1 mark, and dilute with the salt solution of highest strength. Mix and expel the fluid completely into a small test tube. Make similar dilutions with each succeeding strength of salt solution. Place the rack of tubes in the incubator for an hour, or preferably in a water bath at 37 C. for an hour and then note in which tube hemolysis begins, as indicated by the appearance of a red tinge to the supernatant fluid, and in which tube hemolysis is complete, as shown by the total disappearance of the sediment of red cells.

(B). Washed cells. Collect about 5 c.c. of blood by venepuncture in 15 c.c. of 0.5 per cent. sodium citrate solution in 0.9 per cent. sodium chloride solution. Mix thoroughly and centrifugalize. Wash the cells twice with 0.7 per cent. sodium chloride solution and finally draw off the supernatant fluid as completely as possible. To make the test, measure exactly 1 c.c. of each of the hypotonic salt solutions into a series of test tubes and add to each 0.05 c.c. of the corpuscles, using a finely graduated pipette. Allow to stand one hour at 37 C. and read as above. With normal cells hemolysis begins at 0.425 and is complete at 0.350.

Vital Staining.

Puncture the finger and draw a good sized drop of blood into a red cell counting pipette, using the following solution, which must be freshly prepared, as a diluent:

Saturated solution of brilliant cresyl blue in 0.85 per cent. NaCl solution.....5 c.c.

Salt solution, 0.85 per cent.....5 c.c.
Sodium oxalate solution, 2 per cent.....2 c.c.

Add the oxalate to the salt solution, then mix with the staining fluid.

After thoroughly mixing the blood and stain in the pipette allow to stand for ten minutes, then blow the fluid into a centrifuge tube and centrifugalize. Draw off the supernatant fluid until only the sediment of cells remains. Take these up in a capillary pipette and smear on a slightly warmed slide. Allow to dry and examine with the oil immersion lens. (See Vogel and McCurdy, Archives of Internal Medicine, 1913, xiii, 707).

Coagulation Time.

This is determined at the bedside by means of the Boggs instrument. The instrument, microscope, Hagedorn needle, and watch are taken to the bedside. The truncated surface of the glass cone is carefully cleaned and dried, and a drop of water is placed in the bottom of the cell. The blood must be made to flow without much pressure; the first drop is wiped away, and the time that the second appears is noted. The tip of the cone is touched to the drop so that the truncated surface is just covered. The cone is then placed in the top of the cell, and the edge of the drop observed with a number 3 objective, shifting to different parts of the circumference. Air is then blown in, in small gentle puffs, by squeezing the rubber bulb or blowing. At first the cells move freely over each other, then in a mass in a direction parallel to the circumference and later are seen to snap back to their original position. Then, instead of moving in the direction of the circumference, they draw back toward the center of the drop and back toward the edge—radially. When this radial motion is first observed, the end time is noted, the cone is removed, and the drop is touched with a cloth or blotter to make sure of the presence of a coagulum. The time from the appearance of the drop on the finger to the appearance of radial motion is recorded as the coagulation time. A second test should always be made from a fresh puncture. The drop should not be blown upon too frequently or too vigorously. The time varies normally from three to eight minutes.

Formulæ.

Hayem's Solution

Bichloride of mercury	0.5 gm.
Sodium sulphate	5.0 gm.
Sodium chloride	1.0 gm.
Aqua dist.	200 c.c.

Acetic Acid

Two per cent. solution of glacial acetic acid in distilled water; should be filtered every two or three days as a growth of moulds occurs quickly.

Platelet Counting Solution

Number I

Brilliant cresyl blue	1.0 gm.
Distilled water	300 c.c.

This solution is permanent but should be kept in the refrigerator to prevent the growth of moulds.

Number II

Potassium cyanide	1.0 gm.
Distilled water	1400 c.c.

This should be made every nine or ten days. When a count is to be made mix two parts of Number I with three parts of Number II immediately before use, and filter. If allowed to stand exposed to the air a precipitate will form in a short time.

Sodium Carbonate

0.1 per cent. solution in distilled water.

Jenner Stain

One-half gram of powdered stain is dissolved in 100 c.c. of pure methyl alcohol, allowed to stand for several hours, shaken at intervals, and then filtered. It must be kept well corked.

Aqueous Eosin

0.1 per cent. solution of Grübler "w.g." eosin in distilled water.

Methylene Azure

0.25 per cent. solution of methylene azure I in distilled water.

TRANSUDATES AND EXUDATES.

Pleural and peritoneal fluids. Routine examination consists in determination of specific gravity and amount of albumin, and a differential count of the cells.

Specific gravity is determined by a urinometer.

Albumin is determined by Esbach method. Two c.c. of fluid are measured with a pipette into 18 c.c. of distilled water in a graduate and mixed. An Esbach tube is filled to U mark with diluted fluid and to R mark with reagent; then inverted several times. After 24 hours the height of the sediment is read on the scale as grams of albumin to 100 c.c. of fluid.

Differential count is made after centrifuging fluid. The centrifuge tube is tilted and a little sediment is removed on to a warm slide and then spread with another slide like a blood film. It is stained with Jenner or Wood's stain. As a rule a hundred cells are counted, classifying as polynuclears and lymphocytes. The large endothelial cells present in most fluids should be disregarded or separately listed. Smears should be made as soon as possible while fluid is fresh. The cells degenerate as the fluid stands and become difficult to differentiate.

Spinal Fluid. As a routine a total count is made on the freshly shaken fluid and a differential from the centrifuged sediment.

The numerical count should be made as soon as possible after the fluid is drawn. The fluid is shaken gently and a drop placed in a counting chamber, or if bloody, a 1:10 leucocyte pipette is filled to the 0.5 mark with 30 per cent. acetic acid and fluid drawn up to the 11.0 mark, and the cells counted, using a number 6 objective. The dilution may be disregarded unless the count is high, when the total should be increased one-twentieth. Normal fluid contains about eight cells to the cubic millimeter.

Albumin may be roughly estimated by the following device. A narrow test tube about 5 mm. in diameter is strapped with adhesive to the side of an Esbach tube. Fluid is poured in to opposite the U mark, and Esbach's reagent to R mark. The readings are in grams to the liter.

Thick, ropy abdominal (cyst) *fluids* should be tested for mucin and pseudomucin (Wood, p. 651).

Milky, turbid, fluids should be examined for fat, etc., (Wood p. 636). For quantitative determination, see page 85.

Butyric Acid Test for Globulins. (*Noguchi*). To 0.2 c.c. of the spinal fluid (which must be free from blood) in a small test tube add 0.5 c.c. of 10 per cent. butyric acid solution in physiological salt solution, and boil for a few seconds. Add 0.1 c.c. of normal sodium hydrate solution and boil for a few seconds more. A positive reaction is shown by the appearance of a granular or flocculer precipitate, which gradually settles to the bottom of the tube. In strongly positive fluids the precipitate appears in a few minutes, while an hour may be required in specimens containing only a little protein. Reactions occurring after two hours should be disregarded.

URINE.

Routine examination should determine transparency, color, reaction, specific gravity, and presence of albumin or sugar, and should include a microscopical examination.

Transparency. Make note on tag whether urine is turbid (Tr) or clear (Cl).

Color. Note only striking changes from normal pale yellow.

Reaction. Clip with scissors a small square of neutral litmus paper into each cylinder, and leave tag blank (if acid), or mark it N (neutral), or K (alkaline), according to whether the paper is turned red, is unchanged, or is blue, as compared with a slip moistened with distilled water.

Specific gravity. Make bob float freely in cylinder, not touching sides or bottom, read at the bottom of meniscus and mark the last two figures on the tag. If the quantity is insufficient, wait until the other tests have been done and then dilute the urine with an equal volume of water, or if the amount is still too small, with two volumes of water. Determine the specific gravity and multiply the decimal portion of the figure obtained by two, or three, according to the dilution used.

Albumin. Heat and acetic test. Fill row of test tubes in rack two-thirds full of urine, in the same order as specimens are arranged on the table. Add about 5 drops of 2 per cent. acetic acid to each tube, or enough to make the reaction acid, and boil each tube at the top, holding tube at the bottom and directing the flame against the upper portion of the fluid. Add a few more drops of the acid and then examine the tubes by transmitted light against a black background for a cloud in top portion as compared with portion just below it. If there is no clear portion, filter another sample, using Kieselguhr if necessary, acidify and boil, before reporting it negative. If precipitate is flocculent, take tube in holder and heat entire contents to boiling and stand tube in rack above the specimen glass, marking tag "%". When precipitate has settled, fifteen minutes or more afterward, mark the percentage according to the estimated proportion of the column of urine occupied by the sediment. Precipitates too small to settle out are recorded on tag as

"V. f. tr." (very faint trace)

"F. tr." (faint trace)
"Tr." (trace)
"M. tr." (marked trace), or
"V. m. tr." (very marked trace) according to the intensity of the cloud. If the precipitate is indefinite, "V. f. tr.?" may be used, and a sulphosalicylic acid or Spiegler's test made.

Sulphosalicylic Acid Test. Use in cases where heat test is doubtful, or when it is negative and casts are found in the sediment. Stratify urine over 20 per cent. sulphosalicylic acid and look for white ring, or better, add a little of the acid powder to a small tube of urine and boil. Then compare with a control sample in the same size tube and note any cloudiness.

Spiegler's Test. (May be used in same cases). Acidify *strongly* with *glacial* acetic acid and stratify urine over Spiegler's reagent. An opaque white ring appearing immediately indicates an abnormal amount of albumin. Normal urine will show a ring on standing.

Sugar. Fehling's Test. With pipette with rubber nipple put about 1 c.c. of urine in a row of test tubes in the order in which the specimens are arranged on the table, putting three specimens in each test tube, so that the first tube will represent specimens one to three, the second, four to six, the sixth, sixteen to eighteen, etc. Add to each tube about 5 c.c. of boiling Fehling's solution from casserole, and boil each tube, replacing it in the rack. If a red, yellow, or yellowish green precipitate is formed, test the three corresponding specimens separately. If a typical red or golden yellow precipitate is formed, mark tag "sugar"; if only a greenish yellow is obtained, do a phenylhydrazin test, as follows:

Phenylhydrazin Test. Five drops of phenylhydrazin are mixed in a test tube with ten drops of glacial acetic acid and one c.c. of saturated solution of sodium chloride; two or three c.c. of urine are added, and the tube is heated for at least two minutes over the flame. The test tube is allowed to cool for a few minutes, and the crystals which settle to the bottom are removed with a pipette and examined under the microscope.

Benedict's Test. This is more sensitive than Fehling's and also less open to error since the reagent is not reduced by uric acid, creatinine, chloroform, or the simple aldehydes. Into each of a series of numbered test tubes in a rack place five c.c. of the

reagent and eight drops of each of the urines in numerical order. Immerse the rack in a water bath filled with boiling water and keep the water boiling. At the end of exactly five minutes remove the rack and allow the tubes to cool. A positive reaction is shown by the fluid becoming opaque. If no sugar is present the fluid remains clear or only a faint turbidity results due to urates. Large quantities of albumin should first be removed.

Indican. To one-third test tube of urine add an equal volume of Obermayer's reagent, shake, and add 2 c.c. of chloroform. Allow to stand several minutes with occasional shaking. The indican is reported as

"F. tr." (faint trace)
"Tr." (trace)
"M. tr." (marked trace)
"+", "++", and "+++", according to the depth of the blue color.

Blood. Guaiac Test. To about 4 c.c. of urine add 1 c.c. of glacial acid and 2 c.c. of ether; shake gently, pour off the ether, and add a few drops of freshly prepared guaiac tincture and 1 c.c. of hydrogen peroxide. Never use a test tube with yellow copper oxide on its walls resulting from Fehling's or Benedict's sugar tests.

Benzidine Test. Dissolve a knife point of Merck's "Benzidine for Blood Testing" in 2 to 3 c.c. of glacial acetic acid, warming if necessary. Add 10 to 15 drops of the fluid to be tested and then add 3 per cent. peroxide of hydrogen solution drop by drop until a blue or green color appears or 2 c.c. have been added.

Diazo Reaction. Mix 50 c.c. of diazo reagent I with 1 c.c. of diazo reagent II and put about 10 c.c. of the urine with about 10 c.c. of this mixture in a test tube; add 15 to 20 drops of ammonia and shake. A positive reaction is shown by a deep cherry red color in the fluid, and a bright pink shade in the foam. Yellow or orange colors are negative.

N. B. Typhoid urines often contain virulent bacilli.

Acetone. Drop a crystal of sodium nitroprusside in 5 to 10 c.c. of water. Add 1 to 2 c.c. of this solution and a few drops of glacial acetic acid to 5 c.c. of urine, and stratify strong ammonia over the mixture. A purple ring at the junction of the fluids constitutes a positive reaction.

Diacetic Acid. Add a few drops of 10 per cent. ferric chloride

to about 10 c.c. of urine, drop by drop. If a precipitate forms, filter, and add a few more drops of ferric chloride. A "Burgundy" red constitutes a positive reaction. If the patient has been taking phenol, salicylates, aspirin, acetanilid or antipyrin, a dark color will also be produced on the addition of ferric chloride, but it will be a purple rather than a red. In such cases dilute 20 c.c. of the urine with an equal amount of water, boil in a beaker or porcelain dish until the volume has been reduced to the original amount of urine taken and repeat the test. The color due to diacetic acid will be much fainter or will not appear at all, while that due to drugs will not be affected.

Arnold's Test. This is more delicate than the Gerhardt test above, and is not interfered with by drug reactions. Two parts of Arnold's reagent number I and one part of reagent number II are mixed together in a test tube, and an equal bulk of urine is added. Strong ammonia is added, drop by drop. This gives a brown color. An excess of hydrochloric acid is then added, which in normal urine changes the brown to yellow, but in the presence of diacetic acid produces a purple color and on shaking a violet foam.

Bile. 1. Shake urine in test tube to produce foam. A yellow tinge to foam indicates bile. If the acid urine is filtered and fuming nitric acid dropped on the yellow filter paper, a green ring will form about the acid if bile is present. The acidified urine may also be layered over the acid.

2. Layer a very dilute alcoholic solution of iodine over urine. A green ring shows bile. The iodine solution should be of a pale yellow color.

Urobilin. To a test tube containing urine add 25 drops of 10 per cent. zinc chloride and enough ammonia to dissolve the bulk of the precipitate. Filter off the remaining precipitate (phosphates) and hold the test tube against a dark background and in a ray of bright light, such as that of an electric pocket lamp. An excess of urobilin is indicated by a band of green in the fluid.

Urobilin may also be detected by direct examination with a spectroscope. The characteristic band is one between the green and the blue parts of the spectrum. The absorption band is sharper if a few drops of iodine are added to 10 c.c. of urine. The band is marked only when an excess of urobilin is present.

Quantitative Determinations.

Do Quantitative Tests only with Twenty-four Hour Specimens

Quantitative Sugar Determination. Take 25 to 100 c.c. of a twenty-four hour specimen, acidify slightly with acetic acid, and add about 5 grams of powdered normal lead acetate. Filter through dry filter paper. Pour the perfectly clear filtrate into clean tube of saccharimeter till there is a convex meniscus above the top. Slide on cover slip without including air bubble, and screw on cap. Adjust instrument so as to throw daylight through tube. Focus eyepiece so that disc is sharp. Determine zero point without the tube by rotating the analyzer slightly, making the right half disc darker, and then lighter than the left, until the two colors match. Make three readings and average. Insert tube, make three readings, and average, correcting for zero if necessary. The result is per cent. sugar, if rotation is clockwise. Multiply by twenty-four hour amount to obtain grams per day. Do not attempt to read smaller amounts than 0.5 per cent. Oxybutyric acid, if present in large amounts, is sufficiently levorotatory to render polariscopic reading inaccurate. In such cases either use Benedict's quantitative method (see chemical section) or ferment out sugar with yeast, clear, and estimate levorotation. Add this to the reading obtained before fermentation.

To read vernier: I. To the right of O. Read division on primary scale to left of O on the vernier, which is the unit reading. Count to right on vernier scale until line is found which is exactly opposite a line on the primary scale. The number of this vernier line in tenths, (that is, divided by 10) is added to the unit reading obtained from the main scale.

First reading 3; vernier reading 7; result 3.7.

II. To the left of O. Read in same way, but subtract the vernier reading from primary reading.

First reading 3; vernier reading 7; result 2.3.

Quantitative Albumin Determination. Esbach Test. Fill Esbach tube with urine to U mark and with Esbach reagent to R mark. Invert several times so as to mix without shaking and remove any foam with a strip of filter paper. Read in twenty-four hours. The scale shows tenths of 1 per cent. If the albu-

min on boiling is 20 per cent. or more, dilute urine ten times before doing Esbach test.

Total Urea. Doremus Method. Take 15 c.c. of 20 per cent. NaOH and add 35 c.c. of water and 1 c.c. of bromine. Fill Doremus tube and introduce 1 c.c. of urine with special pipette into closed arm of tube, injecting slowly so that bubbles do not escape from bulb. Allow to stand ten minutes and read on scale (milligrams per c.c.). Multiply the result by number of c.c. in twenty-four hour specimen and divide by 1000 to obtain grams per day.

Normal amount, 20 to 30 grams.

The method is extremely inaccurate.

Microscopical Examination.

Place a drop obtained with a pipette from bottom of each specimen glass (or from top layer of urate or pus sediment) on glass plate in order in which the specimens are arranged. Rinse pipette between each specimen to avoid carrying over sediment. Examine each drop carefully under number 3 objective for casts—hyaline, granular, or waxy ("h.c.", "gr.c.", "wax.c."), for cells—epithelial ("ep"), leucocytes ("l"), or red blood cells ("r.b.c."), and for crystals or amorphous deposits—uric acid ("ur. ac."), urates ("ur."), ammonium urate ("am. ur."), amorphous phosphates ("phos."), triple phosphate ("3 phos."), dicalcium phosphate ("ca. phos."), calcium oxalate ("ca. ox."), or other rarer sediments.

Do not report casts without a following "?" unless both ends can be clearly made out to be rounded and fairly abrupt, not tapering. Unless granules are numerous and on focusing appear in the substance of the cast, report cast as "hyaline".

The occurrence of true casts without albumin is rare, and large quantities of albumin rarely occur without either casts, blood, or pus.

The amount of pus is indicated by stating whether it forms a definite sediment or merely a cloud through the specimen, or is only microscopically visible. The presence of even a few polynuclear leucocytes should be reported as pus cells.

Formulæ.

Spiegler's Reagent

Mercuric chloride	80 gm.
Tartaric acid	40 gm.
Glycerine	200 c.c.
Water	2000 c.c.

Benedict's Qualitative Solution

Copper sulphate (cryst.)	17.3 gm.
Sodium or potassium citrate	173.0 gm.
Sodium carbonate (cryst.)*	200.0 gm.
Distilled water to make	1000.0 c.c.

The copper sulphate dissolved in about 100 c.c of water is poured slowly, stirring constantly, into the citrate and carbonate previously dissolved in about 700 c.c. of hot water. The mixture is cooled and diluted to 1 liter.

Fehling's Alkaline Solution

Sodium and potassium tartrate	346 gm.
Sodium hydrate	100 gm.
Water	1000 c.c.

Fehling's Copper Solution.

Copper sulphate (cryst.)	69.278 gm.
Distilled water to make	1000. c.c.

Obermayer's Reagent

Conc. hydrochloric acid (sp. gr. 1.19) containing 2 gm. of ferric chloride to the liter.

Ehrlich's Diazo Reagent. Number I

Sulphanilic acid	1 gm.
Hydrochloric acid (sp. gr. 1.19)	50 c.c.
Water	1000 c.c.

Ehrlich's Diazo Reagent. Number II

Sodium nitrite	1 gm.
Water	200 c.c.

Esbach's Reagent

Picric acid	10 gm.
Citric acid	20 gm.
Water	1000 c.c.

* One-half this amount of the anhydrous salt may be used.

ARNOLD'S REAGENT, SOLUTION NUMBER I

Para-amido-aceto-phenone	1 gm.
Water	100 c.c.
Hydrochloric acid (conc.)	2 c.c.

ARNOLD'S REAGENT, SOLUTION NUMBER II

Potassium nitrite	1 gm.
Water	100 c.c.

EHRLICH'S ALDEHYDE REAGENT

Para-dimethyl-amido-benzaldehyde	15 gm.
Water	270 c.c.
Sulphuric acid (conc.)	30 c.c.

GASTRIC CONTENTS AND STOOLS.

Test Meals. Ewald meals should be sent to the laboratory as soon as expressed. The amount of free acid should be determined immediately; the rest of the examination may be deferred. If a delay is necessary, the specimen should be put on ice. An analysis includes tests for:

1 Total acidity
2 Free hydrochloric acid
3 Combined hydrochloric acid (if free is present)
4 Lactic acid (if free HCl is absent or very low)
5 Blood
6 Rennin
7 Bile.

The contents of the cup are measured and note is made of the color, odor, condition of the bread (whether in fragments as chewed or well macerated) and amount of mucus present (whether well mixed with the meal). Anything unusual in the appearance of the specimen is noted, such as the presence of excess of water, or of undigested egg or other unexpected articles of diet. A drop of sediment is examined under the microscope for food elements, blood cells, pus, yeasts, sarcinæ, and Boas-Oppler bacilli (coarse Gram-positive bacilli, motile, and occurring in chains; very numerous when present). The meal is then carefully strained through two layers of gauze placed in a funnel.

1. *Total acidity.* Ten c.c. are carefully measured with a pipette into a flat porcelain dish, and two or three drops of phenolphthalein added. Decinormal sodium hydrate is then added from

a burette until the red color when spread evenly does not become deeper on addition of another drop. The number of c.c. used is multiplied by 10 and the result used to express the acidity. The usual normal reading is between 40 and 60, but 80 is not abnormal.

2. *Free hydrochloric acid.* Amido-benzol paper is touched with the fluid. If it turns red, free mineral acid is present.

Ten c.c. of the strained juice are measured into another porcelain dish with a pipette and a drop of Töpfer's reagent (dimethyl-amido-azo-benzol) is added. Decinormal soda is then added until the orange-red color changes to a bright yellow—the end reaction. The result is again expressed in the number of c.c. which would be required to neutralize 100 c.c. of gastric contents. The normal amount is given as from 20 to 60, with an average of 40.

If the fluid is small in amount, the free HCl should be determined first, and then a few drops of phenolphthalein added to the same sample and titration continued to determine the total acidity. The amount of alkali used in neutralizing to Töpfer's reagent must of course be included in this reading. If necessary 5 c.c. or even less may be used for titration, but this increases the error.

3. *Combined hydrochloric acid.* This determination can not be made unless there is free HCl present. As an indicator alizarin is used, which reacts to free acid, and to acid phosphates, but not to the loosely combined acid to which phenolphthalein is also sensitive. Ten c.c. of juice with a few drops of alizarin solution are titrated with decinormal alkali. The color changes from yellow to red and then to violet. The end reaction is a violet which is not deepened by the addition of another drop of alkali. The number of c.c. used is subtracted from the number required to neutralize the juice to phenolphthalein, and the difference, multiplied by 10, used to indicate the amount of acid in combination with proteid. Normal readings are from 0.5 to 15 c.c.

4. *Lactic acid.* Specimens with a low free acidity should be tested for lactic acid.

Kelling's Test. Add a few drops of $FeCl_3$ solution to a test tube of distilled water sufficient to produce a very faint yellow. Divide this into two portions and to one add a few drops of the

juice. Lactic acid will produce a distinct "canary yellow" in the tube to which it is added.

5. *Blood.* If brown, questionable particles are seen in the sediment, these should be selected for the test. If not, the fluid should be tested as described under urine. Take 3 c.c. of fluid, 3 c.c. of glacial acetic acid, and 2 c.c. of ether; shake and allow to separate. If large amounts of fat are present extract with neutral ether first. Add 5 to 10 drops of fresh guaiac tincture and 2 c.c. of H_2O_2. A blue color in the ether indicates blood. Benzidine may also be used as described under urine.

6. *Rennin.* Take a few drops of the test meal and add 10 c.c. of milk. If HCl is absent or low in amount mix 1 drop of 1 per cent. calcium chloride solution with the gastric juice before adding it to the milk.

7. *Bile.* A yellow juice may be tested for bile by layering under alcoholic iodine as for urine; a green juice by layering over yellow nitric acid, when a red color may appear from the further oxidation of the biliverdin; but, as a rule, inspection is sufficient.

Vomitus. Examined only as ordered on the tag. The complete routine examination is not necessary.

Stools.

Stools are examined as requested on the tag. For *microscopical examination,* a small particle is taken on platinum loop or glass stirring rod, and, after mixing on the slide with a drop of water, if the stool is solid, is spread in a thin layer under a large cover glass, exerting no pressure. Ova are best found by searching the thin areas with the low power, but it is always advisable to examine a portion of the drop with high power. Two or three drops from different portions of the stool should be examined before a negative report is made.

Muscle fibers are usually bile stained and the preservation of striations is an index of their lack of digestion. Fatty acids occur in sheaves of delicate needles, and fat as refractile droplets which may be identified by adding Scharlach R. Calcium soaps may appear as round bile stained masses. Starch is tested for by running iodine solution under the slide.

Stools for amebas should be sent to the laboratory while still warm and fresh, and examined immediately on the warm stage. While that is being set up they are kept in the incubator. Parti-

cles of bloody mucus should be examined if any can be found. Then smears are made and examined with number 6 objective. *Entameba histolytica* varies greatly in size; it is motile, possesses a distinct ectosarc, and as a rule contains red blood cells. It measures from 30 to 70 micra while *Ameba coli* is usually less than 30 micra.

Stools to be searched for *gall stones, worms,* etc., are placed in gauze, or better, in a fine sieve under a cold water tap in some remote spot. They are washed with occasional stirring and rubbing with a rod or spatula until all soluble and granular portions are washed away. The residue of coarse vegetable fibers, etc., must then be searched.

Blood is tested for by the guaiac or benzidine test (see Urine and Gastric Contents). Solid stools are best rubbed up with a very little water before adding the acetic acid. It should be remembered that meat in the food and iron salts given as medication, will frequently give a positive reaction. The stools should first be extracted with neutral ether; the extract thrown out and the residue treated with acetic acid, extracted and tested in the usual way with guaiac or benzidine.

Bilirubin and *hydrobilirubin* are detected by Schmidt's test. A small amount of stool is placed in a porcelain dish and thoroughly mixed with about an equal volume of saturated solution of bichloride of mercury and allowed to stand for six hours or more. Particles containing hydrobilirubin turn pink or red; those containing bilirubin turn green (biliverdin).

Formulæ.

Phenolphthalein: 1 per cent. solution in 95 per cent. alcohol.

Di-methyl-amido-azo-benzol: 0.5 per cent. solution in 95 per cent. alcohol.

Alizarin: 1 per cent. solution of alizarin S in water; filtered.

Ferric chloride: 5 per cent. solution.

CLINICAL ANALYTICAL METHODS

USE OF THE BALANCE

See that the balance is perfectly level, as indicated by the plumb bob; if it is not, make it so by turning the adjusting screws at the right and left front corners.

Always place the object to be weighed on the left-hand pan, and the weights on the right-hand pan. One reason for this is to avoid errors through possible inequalities in the length of the two arms of the beam. To obtain the true weight of a substance when extreme accuracy is required, counterbalance it with sand or weights and then replace the object with weights; or weigh in one pan and then exchange the substance and weights, weigh again, and take the mean of the weights so obtained.

The beam and pans must always be supported before adding or removing weights, and the weights must be handled only with forceps.

To avoid errors in noting weights always count them twice: 1st, by noting those missing from the box; 2d, by noting the weights as they are taken from the pan and replaced in the box

All objects must be at room temperature when weighed. Warm objects can not be weighed accurately as currents of air are caused which introduce an error.

A platinum crucible should remain in the desiccator ten to fifteen minutes, and a porcelain crucible twenty to twenty-five minutes before weighing.

The supports of beam and pans must be lowered gently to avoid injury to the knife edges.

The balance case must never be left open or with the beam unsupported, and the rider must be removed from the beam.

Be careful to avoid spilling the substance to be weighed on the pans or on the floor of the balance case. If this happens, remove at once by dusting carefully with a camel's hair brush.

Liquids must be weighed in closed weighing bottles, and solids in weighing bottles or watch glasses.

From time to time determine the zero point of the scale. Set the pans swinging by producing a current of air against them by moving the hand palm down briskly downward in front of one pan, and then close the door of the case. Designate the central division of the scale as 10, and determine the points on the scale to which the pointer moves on either side after the first two or three swings. Take at least two readings on one side and one on the other, and obtain the mean; e.g.:

Left	Right
5.6	13.6
6.0	
2)11.6	13.6
5.8	13.6

$5.8+13.6=19.4$

$$\frac{19.4}{2} = 9.7 = \text{zero point}$$

To determine the tenths of a milligram, either use a rider or, if great accuracy be required, estimate them by interpolation, as follows:

Weigh to the nearest milligram too light, and obtain the zero point as described above. Call this reading Y. Add 1 milligram and again obtain the zero point, calling this Y^1. Call the zero point of the balance unloaded A. Then the fraction of a milligram to be added to the weight of the object will be equal to

$$\frac{Y-A}{Y-Y^1}$$

VOLUMETRIC METHODS.

Burettes: With colorless solutions, read the bottom of the meniscus. With colored solutions, such as permanganate, read the top of the column of fluid. To avoid errors through parallax keep the eye at the level of the top of the column of fluid. Solutions should always be at the standard temperature engraved on the volumetric apparatus when they are measured.

Keep burettes covered with caps when not in use, and always use a funnel to fill the burette, but remove it before adjusting the level of the fluid.

As the absolute error of reading remains constant, the percentage of error is greater the smaller the amount of solution used; and the quantity of liquid employed should, therefore, preferably not be less than 20 to 25 c.c.

Burettes and pipettes must be free from grease or the solution will not moisten the surface of the glass evenly and will collect in droplets. To clean burettes and pipettes, use a mixture of 100 c.c. concentrated sulphuric acid to which 1 gram of potassium bichromate has been added. Fill the burettes with the mixture, allow to stand for some time, and then wash carefully in water. Finish by rinsing with distilled water.

Pipettes are ordinarily made to discharge the amount indicated by the graduation; measuring flasks to contain it. In both instances the bottom of the meniscus is the point read.

The opening of the pipette should be small, so that from ten to twenty seconds will be required to empty the instrument. Allow the point to touch the side of the receiving vessel as the last drops flow out.

The upper end of the pipette and the finger used in closing it must be dry in order to control the flow of liquid properly.

If corrosive fluids are to be measured with a pipette, always slip a piece of rubber tubing 20 to 30 cm. in length over the end of the instrument to avoid the possibility of getting fluid into the mouth. Never draw ammonia into a pipette with the mouth.

INDICATORS.

These are bases, acids, or salts, which change color in the presence of acids and alkalies respectively, and possess so intense a color as to enable small amounts to tinge relatively large quantities of fluid.

The indicator itself takes part in the reaction between the acid and alkali; that is, salts are formed or are decomposed into their respective bases and acid radicles. It is this alteration in chem ical constitution that gives rise to the change in color.

The indicators in most common use and their leading characteristics are as follows:

Indicator	Color with Acids	Color with Alkalies	Use with Carbonic Acid in Cold Sol.	Use with Carbonic Acid in Hot Sol.	Use With Ammonium Salts	Use with Organic Acids
Litmus	Red	Blue	Un*	Re.	Re.	Re.
Methyl orange	Pink	Yellow	Re.†	Un.	Re.	Un.
Phenolphthalein	Colorless	Pink	Un.	Re.	Un.	Un.
Cochineal	Purple-red	Blue	Re.	Re.	Re.	Re.
Alizarin red	Yellow	Red	Un.	Re.	Re.	Re.
Congo red	Blue	Red	Un.	Re.	Re.	Un.

STANDARD SOLUTIONS.

As ordinarily employed, the term "normal solution of an acid" is used to indicate a solution of such a concentration as to contain 1 gram of replaceable hydrogen per liter; e.g. normal HCl solution contains the molecular weight of HCl (36.458) per liter; but normal H_2SO_4 contains only one-half the molecular weight, because H_2SO_4 has two replaceable H atoms. Similarly, the concentration of a normal solution of an alkali is such that the amount of the base present corresponds to the amount of acid in the normal acid solution; e.g., normal NaOH contains 40.058 gm. of NaOH per liter, this being its molecular weight, but of sodium carbonate only one-half the molecular weight would be taken, as one molecule of this base is capable of replacing two atoms of acid hydrogen.

Since oxygen (atomic weight 16) is bivalent, oxidizing solutions are made on the basis of 8 grams of available oxygen per liter. For example, in potassium permanganate ($KMnO_4$, molecular weight 158.15) five atoms of oxygen of the eight contained in two molecules of the salt are available for oxidizing purposes; i.e., 80 parts by weight; therefore, one-tenth of this double molecule, (2 x 158.15) or 31.63 grams must be taken for the normal solution, and 3.163 for the decinormal solution.

PREPARATION OF N/10 SULPHURIC ACID.

Take of Merck's anhydrous reagent sodium carbonate about 7 to 8 gm. and ignite gently in a previously weighed platinum crucible, not allowing the heating to exceed a dull red in order to avoid the conversion of small amounts of carbonate into

*Unreliable. †Reliable.

hydroxide, which may take place at high temperatures. The object of the heating is to dehydrate the salt completely and to decompose any bicarbonate which may be present.

Allow to cool in the desiccator, and on the balance quickly remove enough to leave exactly 5.3 gm. in the crucible.

Dissolve this in hot distilled water, rinsing the crucible well. Allow the fluid to cool and then make up to exactly one liter.

Take 7 c.c. of chemically pure H_2SO_4 (sp. gr. 1.84), dilute with four or five volumes of distilled water, and allow to cool.

Transfer to a two liter Giles' flask, and add distilled water to the 2200 c.c. mark. Shake well, and fill a 50 c.c. burette with the acid, and another with the sodium carbonate solution, in each case rinsing out the burette first with some of the solution.

Measure 50 c.c. of the carbonate into a beaker, add a few drops of methyl orange, and titrate with the acid until a pink tinge is noticeable and the addition of a drop of alkali restores the neutral color. Repeat until duplicates are obtained differing by not more than 0.1 c.c.

The acid will be found to be too strong and the amount of water to be added is poured into the flask after reducing the quantity to exactly 2 liters. The amount of water is calculated as follows: for example,

49.5 c.c. of acid neutralizes 50 c.c. of the alkali; then

$$C = \frac{N \cdot d}{n} \qquad C = \frac{2{,}000 \times .5}{49.5} \qquad C = 20.2$$

C = No. of c.c. of water to be added.
N = Amount of solution remaining.
d = Difference between no. of c.c. theoretically required and no. of c.c. actually used.
n = No. of c.c. used in titration.

Repeat the titration and correction until the two solutions are adjusted so as to balance evenly. If the acid is too weak, it is simpler to make it a little too strong again by adding a drop or two of concentrated acid, and then diluting to the required degree.

PREPARATION OF N/10 SODIUM HYDRATE.

Weigh out about 10 gm. of pure NaOH*. Dissolve in water, and dilute to 2200 c.c. in a Giles' flask.

*Merck's reagent sodium hydroxide purified by alcohol is satisfactory.

Standardize with the N/10 H_2SO_4 in the manner described above. For work in which Congo red is to be used habitually it is advisable to employ this indicator in adjusting the alkali to the acid.

Keep in a rubber stoppered bottle.

MELTING POINT DETERMINATION.

Draw a piece of glass tubing into a capillary having a bore of 0.75 to 1. mm.; cut off a portion of this 8 cm. in length; and seal one end. Introduce a small quantity of the substance to be tested by pressing the open end of the tube against the powder and then tapping it down or pushing it in with a fine wire. Attach it to the thermometer with a small rubber band, in such a way that the powder is at the level of the bulb of the thermometer, and the rubber band is as high up as possible. Immerse the bulb of the thermometer in concentrated sulphuric acid in a test tube, which is then heated in sulphuric acid in a beaker of Jena glass or, preferably, in the special Thiele apparatus, and heat with a small Bunsen flame. Watch the substance and note the temperature at which it suddenly becomes transparent or contracts into a droplet. The heating should be slow. If a beaker is used, stirring is necessary to equalize the temperature. At least three determinations should be made.

A somewhat simpler method which is available for many substances is to fill with mercury a porcelain dish about 10 to 12 cm. in diameter. A thermometer is suspended over this in such a way that the bulb is completely immersed in the mercury but does not touch the bottom of the dish. Several particles of the substance under investigation are dropped on the surface of the mercury near the thermometer bulb and the dish is slowly heated with a flame. Watch for the melting point as above.

To correct the temperature for the error due to the exposed portion of the mercury thread, use the following formula:

$S = T+n\ (T-t) \times 0.000159.$

S = Corrected melting point.

T = Observed melting point.

n = Length of exposed portion of thread in degrees.

t = Temperature of air surrounding the exposed air as determined by another thermometer. Ordinarily the room temperature may be used.

SPECIFIC GRAVITY—WESTPHAL BALANCE.

Clean the glass float and platinum wire, by immersing for a moment in concentrated sulphuric acid. Rinse with water, and dry carefully with filter paper.

Fill the cylinder with urine, and bring this to the required temperature of 15 C. by immersing the cylinder in water of a suitable temperature. Adjust the balance with the leveling screw at the foot so that it is in equilibrium with the float hanging in air.

Immerse the float in the cylinder of urine, adjusting the height of the balance so that the float is completely covered, but only a little of the platinum wire dips below the surface of the fluid when the beam is horizontal. Hang the first rider on the same hook as the float, and then bring the balance into equilibrium by hanging other riders on the various notches. The riders on the beam give the specific gravity to four decimals, the integers following in the order of the size of the riders and being indicated by the numbers of the respective notches.

PYCNOMETER.

Weigh the pycnometer empty, and then fill with distilled water at 15 C, drying the outside carefully with filter paper, and weigh again. Weigh again filled with urine at 15 C. Deduct the weight of the instrument in each case, and obtain the specific gravity by dividing the weight of the urine by that of the water.

If only very small amounts of fluid are available, as is sometimes the case in dealing with specimens of urine obtained by catheterizing the ureters a fairly accurate determination of the specific gravity may be made as follows: Into a stoppered weighing bottle of which the weight is known measure with a finely graduated pipette one, two, three, or more c.c. of the fluid in question which is at room temperature, and weigh. Repeat the process with the same amount of distilled water which is also at room temperature. Divide the weight of the urine by the weight of the water. A red cell blood counting pipette may also be used as a pycnometer.

DETERMINATION OF THE NITROGEN PARTITION IN THE URINE.

The chief nitrogenous constituents of the urine are urea, uric acid, ammonia, and creatinine. Under normal conditions these

together make up about 95 per cent. of the total nitrogen of the urine, the balance being composed of small amounts of creatine, amino acids, hippuric acid, allantoin, purin bases, pigments, etc. As carried out for clinical purposes, a complete nitrogen partition includes the determination of the total N and of the amounts of urea, uric acid, ammonia, and creatinine, together with a statement of their nitrogen content, and the percentage which this is of the total N. Ordinarily, however, the factors of greatest interest to the practitioner are the total N, the urea N, and the ammonia N.

Accurately collected twenty-four hour specimens are essential for quantitative determinations on the urine. As a preservative about 10 c.c. of toluol or 5 per cent. alcoholic solution of thymol per liter of urine may be used, and the receptacle should be kept in a cold place. The calculations ordinarily may be simplified by diluting the entire specimen with water to the nearest round number of c.c., as 1000, 1500, etc.

TOTAL NITROGEN DETERMINATION IN URINE.

KJELDAHL METHOD.

Principle: By digestion with concentrated sulphuric acid in the presence of an oxidizing agent, all nitrogenous constituents in the organs, fluids, or excretions of the body are converted into ammonia and the organic bodies decomposed. Potassium sulphate facilitates the reaction by raising the boiling point of the acid. The ammonia is bound by the acid and forms ammonium sulphate. This is then liberated by the addition of alkali and is distilled into a measured amount of decinormal acid. The amount of acid so neutralized may then be determined by titration with decinormal alkali.

Reagents: Concentrated sulphuric acid.
Potassium sulphate.
$CuSO_4$.
30% NaOH.
N/10 H_2SO_4.
N/10 NaOH.
Congo red.
Pumice.

Technique: Measure 5 c.c. of urine with a graduated pipette into a Kjeldahl flask of 800 c.c. capacity. Add 10 c.c. of H_2SO_4

and a small crystal of copper sulphate, and heat over wire gauze in a fume closet until white fumes are given off. Then add a couple of grams of potassium sulphate and continue the heating until the fluid has lost all yellow color, and then for about one-half hour longer. Allow the flask to cool, and carefully and slowly dilute the contents with distilled water to about 400 c.c. Add about a teaspoonful of powdered pumice, and for each 10 c.c. of sulphuric acid used, add 60 c.c. of 30% sodium hydrate without shaking, and avoiding contact of the alkali with the mouth of the flask, as otherwise it is difficult to make the rubber stopper stick tightly. The flask is immediately connected with the safety bulb of the distilling apparatus. An Erlenmeyer flask.* of 500 c.c. capacity has previously been prepared with 50 c.c. of decinormal H_2SO_4, 10 drops of Congo red, and a little distilled water, and adjusted under the condenser so that the tip of the glass safety tube of the latter dips below the surface of the fluid. The distillation is started (at first with a small flame) and continued for at least an hour. The drops falling from the condenser are then tested with litmus paper. If still alkaline, the distillation is continued. Otherwise the receiving flask is lowered, the distillation continued for a few minutes longer, and the flame extinguished. The safety tube is detached and is rinsed with distilled water inside and out into the receiving flask. The fluid is then titrated with N/10 NaOH, the end reaction being a brilliant red. In dealing with untested reagents a blank determination is necessary to determine their possible nitrogen content, and this amount should be deducted from the result.

Each c.c. of N/10 acid neutralized = 0.0017 gm. of NH_3 and 0.0014 gm. of N.

The usual amount of nitrogen on an average diet is 15 to 16 gm. in 24 hours.

UREA DETERMINATION IN URINE.

1. BENEDICT.

Principle: In the presence of potassium bisulphate and in the absence of water, urea is hydrolyzed at 160 C. almost quantitatively into ammonia and carbon dioxide in about an hour, zinc sulphate being added to make the decomposition of the urea complete. Under these conditions uric acid and creatinine are

*Quart milk bottles are cheaper and very satisfactory for this purpose.

not hydrolyzed. The potassium bisulphate, having a replaceable H atom, retains the ammonia, which is then liberated by the addition of alkali and distilled into a known amount of standard acid.

Reagents: Potassium bisulphate.
Zinc sulphate.
Powdered talcum.
30% NaOH.
N/10 H_2SO_4.
N/10 NaOH.
Congo Red.

Technique: Place 5 c.c. of urine (which must not contain sugar) in a wide test tube (preferably provided with an ampulla near the top), add 3 grams of potassium bisulphate, 1.5 grams of zinc sulphate and a small quantity of talcum. Evaporate almost to dryness, either cautiously over a flame or by immersion in a bath of sulphuric acid or paraffin at about 120 C. If the mixture foams badly add a fragment of paraffin the size of a pea. After dryness has been reached, heat the tube in the bath at 162 to 165 C. for one hour. The tube is removed and the contents washed into an 800 c.c. Kjeldahl distillation flask and diluted to 400 to 500 c.c. Add a little talcum or pumice and 10 c.c. of 30% NaOH solution. Distill into 50 c.c. of N/10 H_2SO_4 and titrate as in the Kjeldahl determination (q.v.).

Result: Each c.c. of N/10 NH_3 equals 0.0014 gm. of N, or 3 mgm. of urea. The amount of urea may also be obtained by multiplying the amount of urea N by the factor 2.14. Before calculating the amount of urea N and urea, the N of the preformed ammonia must be determined and deducted.

2. VAN SLYKE AND CULLEN.

Principle: Urease, an enzyme obtained from the soy bean, converts urea into ammonium carbonate. It acts at room temperature, but is most active at a temperature of about 55 C. and in a perfectly neutral solution. It decomposes nothing but urea, and is not interfered with by the presence of glucose. The urea in the urine is converted into ammonium carbonate by the enzyme, the ammonia in the latter is then liberated by the addition of alkali, and is aspirated into a measured

amount of standard acid. The ammonium carbonate formed would by its alkaline reaction retard the action of the enzyme, but the development of alkalinity is prevented by the presence of potassium acid phosphate (KH_2PO_4). An excess of this, however, interferes with the combination of enzyme and urea which precedes hydrolysis of the latter so that it is advisable to use the minimum amount of phosphate that will keep the reaction sufficiently near the neutral point.

Reagents: Soy bean enzyme (Urease)*
0.6% solution of acid potassium phosphate, (KH_2PO_4)
N/50 H_2SO_4
N/50 NaOH
Potassium carbonate solution, 90 grams to 100 c.c. water
Caprylic alcohol†
Alizarin

Technique: Place 5 c.c. of urine in a 50 c.c. volumetric flask and dilute to volume. Pipet 5 c.c. of this diluted urine (equal to 0.5 c.c. of the original) into a wide mouthed test tube, add a soy bean tablet (previously crushed to powder) or 1 c.c. of freshly made enzyme solution, 5 c.c. of 0.6% KH_2PO_4 solution and 2 drops of caprylic alcohol. Connect the tube with the receiving tube prepared as below and allow to stand for 15 to 20 minutes.

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*Either in tablets of 0.1 gm. each containing also 5% of neutral potassium phosphate (K_2HPO_4) to aid in preserving the activity of the solution, or in solution freshly made as follows: solid enzyme, one part by weight in ten of water. First mix to a paste with a little water, then add the rest of the water in portions, forming a cloudy solution.

Standardization of Enzyme. Urease as supplied by the manufacturers varies somewhat in activity, and fresh supplies must always be tested in the following way:

Test solution:

Di-basic potassium phosphate (K_2HPO_4)	43 gm.
Diacid potassium phosphate (KH_2PO_4)	34 gm.
Urea	60 gm.
Water to make	1000 c.c.

Place 5 c.c. of this solution in the tube of the aeration apparatus and bring to exactly 20 C. in a water bath. Add 1 c.c. of 10 per cent. enzyme solution also at 20 C. and allow to stand at this temperature for exactly 15 minutes. Add 6 or 7 grams of potassium carbonate and aerate as usual into 50 c.c. of N/50 acid. The result is expressed in the number of c.c. of N/50 acid neutralized. This should be about 40.

† An efficient antifoaming mixture which is much cheaper than caprylic alcohol may be made as follows:

Diphenyl oxide	60 c.c.
Amyl alcohol	40 c.c.

Into the receiving tube of the aeration apparatus place 25 c.c. of N/50 H_2SO_4 and 2 drops of alizarin. At the end of the 15 minutes allow the air current to pass for half a minute to remove any ammonia that may be free in the urine tube, and then open the latter and introduce 5 c.c. of potassium carbonate solution. Close the tube at once and aerate, first slowly and then more rapidly for fifteen minutes to half an hour, according to the strength of the suction. Then titrate with N/50 NaOH.

Result: The difference between the number of c.c. of acid in the receiver and the amount of alkali needed for neutralization equals the number of c.c. of N/50 NH_3 yielded by the quantity of urine taken. From this result the preformed ammonia (separately determined) must be deducted. Each c.c. of N/50 NH_3 equals 0.00028 of N, and the urea nitrogen multiplied by the factor 2.14 gives the amount of urea. Or, the number of c.c. of acid neutralized by the ammonia from the urine multiplied by 0.056 equals the grams of urea N plus ammonia N per 100 c.c. of urine.

The urea on an average diet is 20 to 40 grams per 24 hours, and equals about 85 to 90 per cent. of the total N.

AMMONIA DETERMINATION IN URINE.

Principle: The ammonia is liberated by the addition of a weak alkali, is removed from the urine by the passage of a strong current of air, and is collected in N/50 sulphuric acid. The amount of the latter neutralized by the ammonia is then determined by titration with N/50 alkali.

Reagents: N/50 H_2SO_4.
N/50 NaOH.
Potassium carbonate solution, 90 grams to 100 c.c. of water.
Alizarin.
Petroleum.

Technique: Use the same apparatus as that employed for the determination of the urea by the urease method. In the receiving cylinder place 25 c.c. of N/50 H_2SO_4 and 3 drops of alizarin. In the aerating cylinder place 5 c.c. of urine, 5 c.c. of potassium carbonate solution, and 1 c.c. of petroleum. Allow the air current to pass first slowly and then more rapidly for about an hour in all. Then titrate back with N/50 NaOH.

Result: Each c.c. of N/50 H_2SO_4 neutralized by the ammonia

liberated corresponds to 0.00028 of N, or 0.00034 of NH_3. In normal urine the average amount of ammonia is about 0.7 grams in 24 hours, or 4 per cent. of the total N.

URIC ACID DETERMINATION IN URINE.
COLORIMETRIC METHOD.

Principle: The uric acid is precipitated by the use of ammoniacal silver solution, the supernatant fluid is discarded, and the precipitate is redissolved with potassium cyanide. The solution is then treated with the phosphotungstic reagent which gives a blue color proportional in depth to the amount of uric acid present; this is compared in the colorimeter with the color produced by a standard solution of uric acid under similar conditions.

Reagents: Ammoniacal silver magnesia mixture.

3% silver lactate ..... 70 c.c.
Magnesia mixture* ..... 30 c.c.
Concentrated aqueous ammonia 100 c.c.
5% Potassium cyanide solution.

Uric Acid Reagent.

Sodium tungstate .....100 gm.
Phosphoric acid, 85%..... 80 c.c.
Water .....750 c.c
Boil for one hour in a flask with a funnel in the neck, cool, and dilute to one liter.

20% Sodium Carbonate Solution.

Uric acid standard solution:

Disodium hydrogen phosphate (cryst.) 9 gm.
Sodium dihydrogen phosphate (cryst.) 1 gm.
Hot water 200 to 300 c.c. If not clear, filter and make up to about 500 c.c. While still hot pour in 200 mgm. of uric acid suspended in a little water in a liter flask. Shake till the uric acid is dissolved, and allow to cool. Add exactly 1.4 c.c. of glacial acetic acid, shake, dilute

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*Magnesia mixture is made as follows:

Crystallized magnesium sulphate ..... 17.5 gm.
Ammonium chloride ..... 35. gm.
Concentrated ammonia .....:..... 60. c.c.
Water to make .....200. c.c.

to the mark, and add about 5 c.c. of chloroform to prevent the growth of moulds. Five c.c. of this solution equals exactly 1 mgm. of uric acid.

Technique: From 2 to 4 c.c. of urine, according to the amount of uric acid expected, is measured into a centrifuge tube, diluted to about 5 c.c. with water and treated with 15 to 20 drops of ammoniacal silver solution. Mix thoroughly with a small stirring rod and allow to stand for ten minutes. Centrifuge, and then pour off the supernatant fluid as completely as possible, inverting the tube over a piece of filter paper. Aspirate the ammonia vapors from the tube by suction with the filter pump and then add to the residue in the tube 2 drops of 5% potassium cyanide solution. Stir well, add 10 to 15 drops of water and stir again. Add 2 c.c. of uric acid reagent, stir, add 10 c.c. of 20% sodium carbonate solution. Allow to stand about half a minute and wash quantitatively into a 50 c.c. graduated cylinder. According to the depth of color as compared with the standard it is diluted to 25 or 50 c.c. The standard is prepared simultaneously by treating 5 c.c. of the standard uric acid solution in a 50 c.c. cylinder with two drops of potassium cyanide solution, 2 c.c. of uric acid reagent, and 10 c.c. of 20% sodium carbonate solution. Dilute to 50 c.c. at the end of a half minute and compare the two in the Duboscq colorimeter.

Result: The calculation is made as follows:

$$X = \frac{S}{R} \times 1 \text{ mg.} \times \frac{Rd}{Sd} \times \frac{Y}{W}$$

X = Mg. of uric acid in 24 hour amount of urine.
S = Reading of Standard.
R = Reading of Unknown.
Rd = Dilution of Unknown.
Sd = Dilution of Standard.
Y = 24 hour volume of urine in c.c.
W = Amount of urine in c.c. taken for determination.

1 mgm. of uric acid equals 0.000333 gm. of N. The amount of uric acid usually present is 0.2 to 1.5 gm. in 24 hours, equivalent to 0.5 to 2.5% of the total N.

TITRATION METHOD.

Principle: The uric acid is precipitated as ammonium urate which is then decomposed by the addition of H_2SO_4; and the liberated uric acid is titrated with N/20 potassium permanganate solution. To remove the mucoid substances always present, which would interfere with the subsequent operations, the urine is first treated with a reagent containing ammonium sulphate and uranium acetate. The resulting precipitate of uranium phosphate carries down the mucoid body. Ammonia is then added to alkalinize the solution and cause the precipitation of the ammonium urate.

Reagents: Solution of uranium acetate, made as follows:

Uranium acetate,	5 gm.
Ammonium sulphate,	500 gm.
Acetic acid, 10%,	60 c.c.
Water,	650 c.c.

Strong ammonia.
Ammonium sulphate solution, 10%.
N/20 potassium permanganate solution.
Strong sulphuric acid.

Technique: Into a tall beaker or cylinder measure 200 c.c. of urine and 50 c.c. of the uranium reagent. Allow to stand for one-half hour, and then decant, siphon, or filter off the supernatant fluid. Measure 125 c.c. (equal to 100 c.c. of urine) of this into a beaker, add 5 c.c. of strong ammonia, and set aside until the following day. Filter off the precipitate and wash with 10% ammonium sulphate solution until the filtrate is nearly or quite free from chlorides. Remove filter from funnel, open and wash the precipitate into a beaker with the ammonium sulphate solution. Add water to make 100 c.c. and dissolve the precipitate with 15 c.c. concentrated sulphuric acid. Titrate at once with the N/20 potassium permanganate solution. The end reaction is the first pink coloration extending through the entire liquid from the addition of two drops of permanganate solution while stirring with a glass rod, and remaining for 30 seconds.

Result: Each c.c. of permanganate solution used corresponds to 3.75 mgm. of uric acid; add 3 mgm. as correction due to solubility of ammonium urate.

1 mgm. of uric acid = 0.000333 gm. of N.

The amount usually present is 0.2 to 1.5 gm. in 24 hours; that is, 0.5 to 2.5% of the total nitrogen.

CREATININE DETERMINATION IN URINE.

Principle: On adding picric acid and sodium hydrate to a solution containing creatinine, a deep red color is produced. The intensity of this in the specimen of urine is compared with that of a standard solution of potassium bichromate. Sugar and albumin do not interfere; but acetone and diacetic acid, if present, must be removed by heating.

Reagents: N/2 potassium bichromate solution (24.55 gm. per liter)
Sat. picric acid solution (about 12 gm. per liter)
10% NaOH.

Technique: Ten c.c. of urine is measured into a flask with a mark at 500 c.c., 15 c.c. of picric acid solution and 5 c.c. of sodium hydrate are added, and the mixture allowed to stand for five minutes. Pour a little bichromate solution into the two cylinders of the Duboscq colorimeter and set the left hand one to the 8 mm. mark. Then make several readings in order to accustom the eye to the colors. Now dilute the urine mixture to the 500 c.c. mark, and rinse out and half fill one of the cylinders with it, wipe off the glass rod, and then make several readings immediately and take the average. The reading must be made within ten minutes.

If the urine contains more than 15 mgm. or less than 5 mgm. of creatinine, repeat the determination with a smaller or larger amount of urine, as outside of these limits the determination is less accurate.

Calculation: $$10 \times \frac{8.1}{\text{Reading}} = \text{mgm. creatinine.}$$

1 mgm. creatinine = 0.000371 gm. N.

The normal excretion of creatinine is about 20 to 30 mgm. per kilo of body weight, fat persons yielding less and thin persons more. On an average diet the creatinine nitrogen equals about 3 to 5 per cent. of the total nitrogen.

CREATINE DETERMINATION IN URINE.

Principle: On heating creatine with dilute mineral acids it is dehydrated and its anhydride creatinine is formed. At a temperature of 117 to 120 C. the conversion is complete in fifteen minutes. This temperature is reached when the pressure is one kilo per square centimeter, or 14 pounds per square inch.

Technique: Place 20 c.c. of the urine in a 500 c.c. Erlenmeyer flask, add the same amount of normal hydrochloric acid, and heat in the autoclave for twenty to thirty minutes at 117 to 120 C. Cool and make the volume up to exactly 50 c.c. with distilled water, shake thoroughly, and measure off 25 c.c. of the mixture, corresponding to 10 c.c. of the original urine. Neutralize this with 10 c.c. normal sodium hydrate solution, and then determine the creatinine by the method described above. From the amount of creatinine so obtained deduct the amount of creatinine determined in the unheated urine. The difference will be the creatine content of the original urine in terms of creatinine. To obtain the amount of creatine multiply this figure by the factor 1.16. The dark color produced by the heating usually causes no difficulty, owing to the dilution necessary in making the mixture for the colorimeter.

MISCELLANEOUS DETERMINATIONS.

DETERMINATION OF CHLORIDES IN URINE.

Principle: The chlorides in a definite amount of urine are precipitated by a standard solution of silver nitrate in the presence of an excess of free nitric acid. The precipitate of silver chloride is filtered off, and the excess of silver remaining in solution is determined in the filtrate by titrating with a standard solution of potassium sulphocyanide, using a solution of iron alum as an indicator. As soon as the sulphocyanide has combined with all the silver to form white silver sulphocyanide, the deep red ferric sulphocyanide is formed and indicates the end reaction. In this way the amount of silver solution which combined with the chlorides is ascertained. Albumin need not be removed unless present in large amount.

Reagents: $AgNO_3$ solution, of which 1 c.c. equals 0.01 NaCl.*

Potassium sulphocyanide solution, of which 1 c.c. equals 1 c.c of the $AgNO_3$ solution.

Saturated solution of ammonioferric alum.

Nitric acid.

Technique: Ten c.c. of urine, accurately measured with a pipette, is placed in a flask with a mark at 100 c.c.; about 50 c.c. of water, 5 c.c. of nitric acid, and 20 c.c. of the silver solution are added. The nitric acid is to be measured in a graduate, but the silver solution must be accurately measured.

The mixture is well shaken, and distilled water is added to the 100 c.c. mark. The fluid is filtered through a small dry filter, and 50 c.c. taken, this amount corresponding to 5 c.c. of urine. This 50 c.c. is then poured into a beaker and the measuring flask rinsed into the same with distilled water.

Five c.c. of the alum solution is added, and the whole is titrated with the sulphocyanide solution to the appearance of the first reddish tinge.

Calculation: 20 minus the no. of c.c. of sulphocyanide solution used equals the no. of c.c. of silver solution required to precipitate the chlorides. Each c.c. of silver solution equals 0.01 of NaCl; therefore, 20 minus twice the reading, x 10 = milligrams of NaCl in 10 c.c. of urine. The usual amount in 24 hours is 10 to 15 grams.

DETERMINATION OF PHOSPHATES IN URINE.

Principle: The urine is first treated with a solution of sodium acetate and glacial acetic acid in order to convert any monacid phosphate into diacid phosphate, and also to neutralize any nitric

*The silver nitrate solution should contain 29.059 gm. of $AgNo_3$ per liter, and the solution of potassium sulphocyanide equivalent to this requires 16.62 gm. per liter. As the latter salt is hygroscopic, the exact amount cannot be weighed accurately, so weigh out about 22 gm. and dissolve in 1200 c.c. of water. Pipette 10 c.c. of the silver solution into a porcelain dish, dilute with 100 c.c. of distilled water, acidify with nitric acid and add 5 c.c. of ammonioferric alum solution. Titrate with the sulphocyanide solution to the appearance of a permanent reddish color and then dilute the sulphocyanide solution to the proper degree according to the principles given on page 44.

To check the accuracy of the silver solution, dry chemically pure sodium chloride at 120 C. and weigh out exactly 0.150 gm. Dissolve in 100 c.c. of distilled water, add a few drops of 5 per cent. potassium chromate solution, and titrate with the silver solution till an orange tint appears. Exactly 15 c.c. of the silver solution should be necessary.

acid that may be formed during the subsequent titration, as this, if allowed to remain free, would cause partial solution of the precipitated uranyl phosphate. The titration is then performed with a standard solution of uranium nitrate which gives with phosphoric acid compounds in acetic acid solution a yellowish white precipitate of uranyl phosphate (UO_2HPO_4). As indicator cochineal is used, which gives a green color in the presence of an excess of uranium solution. Sugar and albumin do not interfere with the method. If the urine is deeply bile stained, it should be acidified with HCl and decolorized by the addition of a few crystals of potassium permanganate.

Reagents: A solution of uranium nitrate of which 20 c.c. equals 0.1 gm. P_2O_5.

Acetic acid solution: Sodium acetate, 100 gm.
Glacial acetic acid, 30 gm.
Water to 1000 c.c.

Tincture of cochineal.

Technique: Fifty c.c. of filtered urine is treated with 5 c.c. of the acetic acid mixture. A few drops of the cochineal indicator are added. The fluid is heated to boiling and titrated with the uranium solution until a trace of green color is seen, which does not disappear on stirring. The titration is repeated until accurately corresponding duplicates are obtained, and the result is calculated as follows:

20 : 0.1 : : n : x, n being the number of c.c. of uranium solution used.

The percentage of P_2O_5 equals 2x.

The usual amount of P_2O_5 on an average diet equals 1 to 5 gm. in twenty-four hours.

DETERMINATION OF SULPHATES IN URINE.

Sulphur occurs in the urine in the following forms:

1 Inorganic or preformed sulphates.
2 Ethereal or conjugated sulphates in which H_2SO_4 is combined with aromatic compounds.
3 Neutral or unoxidized sulphur.

The partition of the three forms is determined as follows. The urine must be freed from albumin.

BENZIDINE METHOD.

Principle: The sulphates are precipitated as an insoluble salt of benzidine (p-diaminodiphenyl, $NH_2 . C_6H_4 . C_6H_4 . NH_2$) and this is then dissociated by the addition of a volumetric solution of sodium hydrate.

Reagents: Benzidine solution.*
Dilute HCl (1 part concentrated HCl to 4 parts H_2O by volume)
Water saturated with benzidine sulphate†.
N/10 NaOH.
Phenolphthalein.

INORGANIC SULPHATES.

Technique: Into a 250 c.c. Erlenmeyer flask pipette 25 c.c. of urine and add dilute HCl until it is distinctly acid to Congo red paper (usually 1 to 2 c.c.). Add 100 c.c. of the benzidine solution and allow to stand for ten minutes. The precipitate is filtered off by suction using a small Büchner funnel or a funnel with a perforated porcelain filter plate, or with a platinum filtering cone. Do not allow the precipitate to be sucked dry. Wash the precipitate with water saturated with benzidine sulphate until the filtrate gives no reaction with Congo red (10 to 20 c.c.). Transfer precipitate and paper back to the original flask with about 50 c.c. of water and titrate hot with N/10 NaOH and phenolphthalein until pink.

Result: Multiply the number of c.c. of N/10 alkali used by

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*Rub into a paste with about 10 c.c. of H_2O 4 grams of Kahlbaum's or Merck's benzidine. Transfer this paste with 500 c.c. of H_2O to a two liter volumetric flask, add 5 c.c. of concentrated HCl (Sp. gr. 1.19) and shake until dissolved. Finally dilute to 2000 c.c. 150 c.c. of this solution, which keeps indefinitely, precipitates 0.1 gm. of H_2SO_4.

†Preparation of Benzidine Sulphate. 18.4 gm. of p-benzidine are dissolved in 50 c.c. of alcohol, filtering if solution is not complete. 5.7 gm. of concentrated (95%) sulphuric acid (sp. gr., 1.84) measured in a graduated pipette and also dissolved in 50 c.c. of warm alcohol is slowly added to the benzidine solution with constant stirring. When the precipitate has settled, test the supernatant alcohol with moistened blue litmus paper, adding a little more alcoholic acid if necessary to insure a slight acidity. The grayish white benzidine sulphate is then filtered on a Büchner funnel, using suction, washed with alcohol several times, once with ether, and dried on a water bath. If the precipitate appears to be at all lumpy, it is best to grind it thoroughly in a porcelain mortar before filtering and washing.

Yield, 28 gm.

.004 to obtain the number of gm. of SO_3 present in 25 c.c. of urine.

TOTAL SULPHATES.

Principle: The ethereal sulphates are split by boiling with HCl and the total sulphates resulting are determined just as above.

Technique: Twenty-five c.c. of urine and twenty c.c. of dilute HCl are gently boiled in an Erlenmeyer flask for 15 to 20 minutes. Cool, neutralize with sodium hydrate, then make acid to Congo red with dilute HCl and proceed as above.

ETHEREAL SULPHATES.

The amount of these is obtained by subtracting the amount of the inorganic sulphate from that of the total sulphate.

TOTAL SULPHUR.

Reagents: Those used above.

Benedict's solution:

Crystallized copper nitrate,	200 gm.
Sodium or potassium chlorate,	50 gm.
Distilled water to	1000 c.c.

Principle: All of the sulphur present is oxidized by heating with a reagent composed of copper nitrate and potassium chlorate. The former on heating decomposes into two vigorous oxidizing agents: nitrogen dioxide and cupric oxide, the latter forming a stable compound with the oxidized sulphur. This is dissolved in dilute hydrochloric acid, and the sulphur precipitated with benzidine as above.

Technique: Ten c.c. of urine is measured into a small (7 to 8 cm.) porcelain evaporating dish, and 5 c.c. of the reagent added. The contents of the dish are evaporated over a free flame, which is regulated to keep the solution just below the boiling point, so that there can be no loss through spattering. When dryness is reached the flame is raised slightly until the entire residue has blackened. The flame is then turned up in two stages to the full heat of the Bunsen burner and the contents of the dish thus *heated to redness for ten minutes after the black residue* (which first fuses) *has become dry.* This heating is to decompose the last traces of nitrate and chlorate. The flame is then removed and the dish allowed to cool more or less completely, 10 to 20

c.c. of dilute (1:4) HCl is next added to the residue in the dish, which is then warmed gently until the contents have completely dissolved and a perfectly clear, sparkling solution is obtained. This dissolving of the residue requires scarcely two minutes. With the aid of a stirring rod the solution is washed into a small Erlenmeyer flask, is neutralized with sodium hydrate, made acid to Congo red with dilute HCl, and then precipitated with benzidine as above.

The total sulphur per 24 hours usually varies from 1 to 3.5 gm. of SO_3. Of this the inorganic sulphates are about 85 to 90%; the ethereal sulphates, 8 to 10%; and the neutral sulphur about 5%, but the sulphur partition is largely modified by changes in diet.

BARIUM CHLORIDE METHOD.

Principle: The sulphates in the urine are precipitated by the addition of an excess of barium chloride solution. The precipitate of barium sulphate is filtered off, washed, dried, ignited and weighed.

Reagents: Dilute HCl (1 part concentrated HCl to 4 parts H_2O by volume)
Barium chloride solution, 5 per cent.

INORGANIC SULPHATES.

Technique: Into an Erlenmeyer flask place about 100 c.c. of water; 10 c.c. of dilute hydrochloric acid, and 25 c.c. of urine. If the urine is dilute take 50 c.c. instead of 25, and a correspondingly smaller amount of water. Ten c.c. of barium chloride solution is added drop by drop from a pipette having a short piece of rubber tubing slipped over its upper end and provided with a screw pinchcock. The urine must not be disturbed while the barium chloride is being added. At the end of an hour or later, the mixture is shaken and filtered through a weighed Gooch crucible, as described below. The precipitate is washed with at least 200 c.c. of water. The crucible is then dried, ignited, placed in the desiccator, cooled, and then weighed.

The increase in weight indicates the amount of $BaSO_4$ referable to the inorganic sulphates in the amount of urine used.

1 gm. $BaSO_4$ = 0.34301 gm. SO_3.
1 gm. " = 0.42015 gm. H_2SO_4.
1 gm. " = 0.13744 gm. S.

To express the results in terms of SO_3, H_2SO_4, or S, multiply the weight of $BaSO_4$ by the proper factors as given above.

TOTAL SULPHATES.

Principle: The ethereal sulphates are split by boiling with HCl, and the total sulphates resulting determined just as above.

Technique: Twenty-five c.c. of urine and 20 c.c. of dilute HCl (or 50 c.c. of urine and 4 c.c. of concentrated HCl) are gently boiled for 20 to 30 minutes in an Erlenmeyer flask, into which a funnel has been placed to reduce the loss of steam. The flask is cooled for about 2 to 3 minutes in running water, and the contents are diluted with cold water to about 150 c.c. Then precipitate with barium chloride, filter through a Gooch, and weigh as above.

ETHEREAL SULPHATES.

The amount of these may be obtained by subtracting the amount of inorganic sulphates from that of the total sulphates.

TOTAL SULPHUR.

Oxidize the sulphur with Benedict's sulphur reagent as described under the benzidine method. The solution obtained by dissolving the residue in the porcelain dish is washed quantitatively into a small Erlenmeyer flask, diluted with cold distilled water to 100 to 150 c.c., 10 c.c. of 5 per cent. barium chloride solution is added drop by drop and the solution is allowed to stand for about an hour. It is then shaken up and filtered as usual through a weighed Gooch filter.

To make the Gooch crucible filter, pour a suspension of asbestos fiber in water into the crucible while strong suction is being applied, so that a firm felt work (about 2 mm. thick) is formed. The asbestos is prepared by scraping the crude material with a knife and adding the fibers to a large bulk of 5% HCl in a cylinder. Air is blown through to separate the fibers thoroughly, and the mixture is allowed to settle for a few minutes. The upper portion of the fluid containing the finer fibers is decanted and kept separately from the lower.

In making the filter the coarse material is poured on first and a little of the fine afterward. The filter is then washed by drawing distilled water through in a slow stream, is dried at 120 C., ignited, and weighed.

In igniting the barium sulphate precipitates the flame must not be applied directly to the bottom of the crucible, in order to avoid mechanical losses. The crucible is placed on a platinum crucible lid on a pipe stem triangle, and the outer, oxidizing flame of the Bunsen burner is used first gently and finally with full force.

The results obtained by these procedures will be in terms of barium sulphate. It is customary to report them in terms of SO_3. This is done by multiplying the amount of $BaSO_4$ by the factor 0.34301.

TOTAL ACIDITY DETERMINATION IN URINE.

Principle: Determination of the acidity of the urine by direct titration is not possible owing to the occurrence of calcium and ammonium salts in the presence of monobasic phosphates. The addition of potassium oxalate, however, overcomes these difficulties by holding in solution the di- and tricalcium phosphates and preventing dissociation of the ammonium compounds.

Technique: To 25 c.c. of urine add 15 to 20 gm. of powdered potassium oxalate and several drops of phenolphthalein.

Shake for several minutes, and titrate at once with N/10 sodium hydrate until a distinct permanent pink is obtained, shaking the flask during the titration.

To neutralize a 24-hour amount of urine from 550 to 650 c.c. of N/10 alkali are usually required.

FREEING URINE FROM ALBUMIN AND THE GRAVIMETRIC DETERMINATION OF ALBUMIN.

Take 100 c.c. of urine. If necessary make it faintly acid with dilute acetic acid, and heat on the water bath until the albumin begins to separate in flakes. After drying the outside of the beaker boil for two minutes over a free flame.

If the albumin does not coagulate well, carefully add a drop or two of dilute acetic acid. Excess of acid may cause some of the albumin to stay in solution.

Filter while still hot through a Schleicher and Schüll ash-free filter (No. 589, 9 cm.) that has previously been dried at 120 C. and weighed. If further quantitative determinations are to be made on the urine, filter into a measuring flask and wash out the beaker and filter with small amounts of distilled water until the volume at room temperature is brought up to its original

amount. Wash precipitate on the filter with more warm water and then with alcohol followed by ether.

Dry in the hot air oven at 120 C. to constant weight.

If great accuracy be desired, fold the filter carefully with the precipitate inside, wrap a platinum wire about it, hold it over a weighed platinum crucible and burn the filter paper, allowing all the ash to fall into the crucible. Then ignite, weigh again, and take the weight of the ash from the weight of the dried substance.

The albumin may also be removed before doing a nitrogen partition by adding an equal volume of alumina cream* and filtering.

DETERMINATION OF GLUCOSE IN URINE.

BENEDICT.

Principle: Through the substitution of sodium carbonate for the strong alkali in Fehling's solution, Benedict's solution is made much more sensitive as a reagent for glucose. The reduced copper produced when the solution is boiled in the presence of glucose is precipitated not as the red suboxid (Cu_2O), but as the white cuprous sulphocyanate (CuSCN) which makes it easy to determine when the last trace of blue has been removed from the solution, showing complete reduction of all the copper.

Reagents: Benedict's quantitative solution†
Sodium carbonate, crystallized.
Powdered pumice.

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*The aluminum hydroxide for this purpose is made as follows: To a 1 per cent. solution of ammonium alum add a slight excess of 1 per cent. solution ammonium hydroxide at room temperature. Wash the precipitate by decantation until the wash water gives a very faint residue on evaporation (Tracy and Welker).

† Benedict's quantitative solution is made as follows:

	gm. or c.c.
Copper sulphate (pure crystallized)	18.0
Sodium carbonate (crystallized)	200.0
Sodium or potassium citrate	200.0
Potassium sulphocyanate	125.0
5% potassium ferrocyanide solution	5.0
Distilled water to make a total volume of	1000.0

With the aid of heat dissolve the carbonate, citrate, and sulphocyanate in enough water to make about 800 c.c. of the mixture, and filter if necessary. Dissolve the copper sulphate separately in about 100 c.c. of water and pour the solution slowly into the other liquid, with constant stirring. Add the ferrocyanide solution, cool and dilute to exactly 1 liter. Of the various constituents, the copper salt only need be weighed with exactness. Twenty-five c.c. of the reagent are reduced by 50 mgm. of glucose.

Technique: Twenty-five c.c. of the reagent is measured with a pipette into a porcelain evaporating dish, and 10 to 20 gm. of crystallized sodium carbonate or one-half the weight of anhydrous sodium carbonate are added, together with a little pumice stone or talcum. The mixture is heated over a free flame until the carbonate is dissolved and then the urine, diluted 1:10, (unless the sugar content is expected to be very low) is run in from a burette rather rapidly until the entire disappearance of the blue color, which marks the end point. During the entire titration the fluid must be kept boiling vigorously and the water lost by evaporation may be replaced from time to time.

Result: If the urine was diluted 1:10, the percentage of sugar may be obtained as follows:

$$\frac{0.050}{X} \times 1000 = \text{percentage of sugar in undiluted urine, X being the number of c.c. of diluted urine required to produce the end reaction.}$$

DETERMINATION OF ACETONE AND DIACETIC ACID IN URINE.

Principle: The pre-formed acetone and that developed from diacetic acid by heating is aspirated into a known amount of hypoiodite solution. The iodine unites with the acetone to form iodoform, and by titration with thiosulphate solution the amount of the former remaining uncombined is ascertained. By subtracting this from the amount originally taken the amount entering into combination with the acetone is learned and the quantity of the latter may be determined by calculation.

Reagents: N/10 Iodine solution*.
N/10 Sodium thiosulphate solution†.
40% potassium hydroxide solution.
10% phosphoric acid.
Sodium chloride.
Kerosene.
Starch solution (1% solution of soluble starch with one drop of cinnamic aldehyde per 100 c.c.)

*N/10 iodine solution contains 12.692 gm. iodine to 1000 c.c. To make it dissolve in a liter flask 20 gm. of pure potassium iodide in a little water. Add 12.8 gm. of sublimed iodine and shake gently until dissolved. Dilute to 1000 c.c. If necessary the solution may be filtered through asbestos. Standardize against N/10 sodium thiosulphate solution by titrating 25 c.c.

Technique: Into the receiver of the aeration apparatus introduce 150 c.c. of water, 10 c.c. of 40% potassium hydroxide solution and exactly 50 c.c. of N/10 iodine solution. Use a wide test tube as an aeration cylinder and arrange this so that heat may be applied during aeration. Into the tube place 20 c.c. of the urine to be examined, 10 drops of 10% phosphoric acid, 10 gm. of sodium chloride (acetone is insoluble in saturated salt solution) and a little kerosene to prevent foaming. It is well to attach a wash bottle containing hypoiodite solution to the other side of the aeration tube in order to free the in-coming air from possible traces of substances capable of combining with the iodine. The apparatus is connected with a pump and air is allowed to pass for half an hour or more, according to the strength of the air current. During this period the urine mixture is heated just to the boiling point and after an interval of five minutes is again heated in the same manner. The fluid in the receiving cylinder is acidified with concentrated hydrochloric acid, an excess of iodine solution being shown by the development of a brown color. Add a little starch solution and titrate with N/10 sodium thiosulphate until the blue color disappears. The end point may be determined more accurately if the starch is not added until the titration is almost finished, as shown by the brown color becoming paler.

Result: X = (A-Y) x 0.967.

A = number of c.c. of iodine solution in receiving cylinder.

Y = number of c.c. of thiosulphate solution used in titrating.

X = mg. of acetone (pre-formed and that from diacetic acid) in the amount of urine used.

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of iodine solution with the thiosulphate until the yellow color of the solution is just discharged by one drop of thiosulphate.

†N/10 sodium thiosulphate solution contains 24.824 gm. of the salt ($Na_2S_2O_3$-5 H_2O) to 1000 c.c. To make it dissolve 30 gm. of the crystalline salt in 1000 c.c. of water. Standardize with potassium acid iodate as follows; Dissolve in a little water exactly 0.1625 gm. of pure potassium acid iodate. Add about 1 gm. of potassium iodide and a few c.c. of dilute HCl. After a few minutes titrate with the thiosulphate solution so that the final yellow color of the fluid is just discharged by one drop of the thiosulphate solution. This is then diluted so that the volume consumed in titration shall measure exactly 50 c.c.

One c.c. of N/10 iodine solution is equivalent to 0.967 mgm. of acetone.

DETERMINATION OF ACETONE AND OXYBUTYRIC ACID IN URINE.

SHAFFER AND MARRIOTT

"The oxidation method, combined with the preliminary distillation for the removal and determination of acetone and diacetic acid, is carried out as follows: From 25 to 100 c.c. or more of urine (usually 50 c.c.) are measured with a pipette into a 500 c.c. volumetric flask containing 200 c.c. to 300 c.c. of water. Basic lead acetate solution (U. S. P.) is added in amount equal to the urine used* and the liquid well mixed. Strong ammonia water, about half the volume of the lead acetate, is next poured in the flask, diluted to the mark with water, shaken, and after a few minutes' standing, the liquid is filtered, preferably through a folded filter, 200 c.c. of the filtrate is measured into a round bottom flask (800 c.c. or liter Kjeldahls are convenient) diluted with water to about 600 c.c., 15 c.c. of concentrated sulphuric acid and talc or boiling stone added, and the mixture distilled until about 200 c.c. of the distillate have collected (Distillate A).

"The distilling flask must be fitted with a dropping tube and water run in from time to time to prevent the volume in the flask from becoming less than 400 to 500 c.c.

"Distillate A, which contains the acetone preformed and aceto-acetic acid, and which should be collected in a second Kjeldahl flask, is redistilled (for about twenty minutes) after adding 10 c.c. of 10 per cent. sodium hydroxide†. The distillate so obtained (A_2) is titrated with standard iodine and thiosulphate solutions.

"The residue of urine plus sulphuric acid from which Distillate A was obtained is again distilled‡ dropping in either water, when necessary to keep the volume between 400 and 600 c.c., or a dilute solution of potassium bichromate. From 0.5 to 1 gram bichromate will usually be sufficient, and not more than 1 gram should be added unless the liquid turns green indicating a great reduc-

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*If the urine contains but little or no sugar only half the amount or less of lead acetate should be used.

† In many instances, when a high degree of accuracy is not required, this redistillation may be omitted, and "distillate A" titrated direct; the results so obtained are slightly higher than those after redistillation from alkali.

‡ The distillation is actually not interrupted; after "A" has collected, a new receiving flask is adjusted and bichromate solution slowly added through the dropping tube. The receiving tube of the condenser must dip below the surface of the water in the receiving flask.

tion to chromium sulphate; very rarely 2 or 3 grams of bichromate may be necessary, especially if the sugar has not been completely removed.

"A 10 per cent. solution of potassium bichromate is kept on hand and 10 c.c. of this, diluted to 100 c.c., are measured out for each determination. 20 c.c. of the dilute solution (0.2 gram $K_2Cr_2O_7$) are first added slowly through the dropping tube and then 10 c.c. portions every fifteen or twenty minutes until the whole has been added. Should the liquid become markedly green the bichromate must be added at correspondingly shorter intervals and in amount sufficient to maintain a slight red-yellow color of the chromic acid, which may be detected even in the presence of the green. The distillation is continued with moderate boiling for from two to three hours. The distillate (B), which should be collected in a liter flask to avoid transference, is again distilled for about twenty minutes after adding 10 c.c. of 10 per cent. sodium hydroxide and 25 c.c. of 3 per cent. hydrogen peroxide. The flask must be heated cautiously until the peroxide has decomposed. This distillate (B_2) is titrated with the standard iodine and thiosulphate.

$$1 \text{ c.c. of } \frac{N}{10} \text{ iodine equals } 0.968 \text{ mgm. acetone equals } 1.736 \text{ mgm.}$$

oxybutyric acid, or

$$1 \text{ c.c. of } \frac{1.035N}{10} \text{ iodine (equals } 13.13 \text{ mgm. } I_2\text{) equals } 1 \text{ mgm.}$$

acetone equals 1.793 mgm. oxybutyric acid."

The results for oxybutyric acid obtained by this method are uniformly 5 to 10 per cent. too low, owing to the fact that in the oxidation with bichromate some of the acetoacetic acid undergoes acid decomposition with the formation of acetic acid instead of acetone, and a correction of 10 per cent. should therefore be made.

AMYLASE DETERMINATION IN URINE OR OTHER FLUIDS. WOHLGEMUTH.

Principle: A series of dilutions of the fluid to be tested is made and the same amount of starch solution is added to each. After warming the tubes to about 38 C. for half an hour the

action of the enzyme is checked by immersion in ice water and the dilution of the fluid still containing enough enzyme completely to digest the starch to the dextrin stage is determined by the addition of iodine solution to each tube.

Reagents: 0.1% starch solution*.
N/50 iodine solution, freshly prepared; made from a stock solution.

Technique: To each of ten test tubes in a rack add 1 c.c. of normal saline solution, except to the first, in which place 1 c.c. of the fluid to be tested. With a 1 c.c. pipette graduated in one-tenths add one c.c. of the fluid to be tested to the second tube, thoroughly mixing the two tubes. From this tube take 1 c.c. of the mixture and place in tube 3, continuing the process of mixing and withdrawing 1 c.c. from each tube in the series. The one c.c. withdrawn from the last tube is discarded. In this way the following series of dilutions is produced:

No. 1 = 1 c.c.	No. 6 = 0.032
No. 2 = 0.5	No. 7 = 0.016
No. 3 = 0.25	No. 8 = 0.08
No. 4 = 0.125	No. 9 = 0.004
No. 5 = 0.0625	No. 10 = 0.002

To each tube add two c.c. of 0.1 per cent. starch solution, mix, and place rack in the water bath at exactly 38 C. for exactly half an hour. The temperature must not vary more than one-half a degree above or below 38 C. The tubes are then removed from the water bath and placed at once in ice water to inhibit further action for five minutes. Two or more drops of N/50 iodine solution, as may be needed to bring out a permanent color, are added to each tube, taking care to use exactly the same number of drops in each case. The reading is made from the tube in which all the starch has been converted to the dextrin stage, that is, in which the iodine produces no shade of blue. To determine this, select the first tube in which any blue tone is appreciable and take the tube next preceding as the one showing the end reaction.

Result: The result is expressed in terms of the number of c.c. of 0.1% starch solution digested by 1 c.c. of the fluid to be tested

*Made from Kahlbaum's soluble starch. It should be freshly prepared every three days and kept in the refrigerator.

under the conditions of the determination. It is calculated as follows:

$$X = \frac{S}{U}$$

X = c.c. of 0.1 per cent. starch solution digested by one c.c. of the fluid tested.

U = c.c. of the fluid to be tested contained in tube showing end reaction.

S = c.c. of starch solution digested in tube showing end reaction.

Reports are made as follows:

$d\,\frac{38°}{30} = X$, or as $D\,\frac{38°}{24} = X$, according as digestion is allowed to go on for thirty minutes or twenty-four hours at the temperature stated.

Normal urine usually gives a value of about 16.

PHENOLSULPHONEPHTHALEIN TEST FOR RENAL FUNCTION. ROWNTREE AND GERAGHTY.

Principle: A measured amount of a dye which under normal conditions is very rapidly excreted by the kidneys is injected subcutaneously. The amount present in the urine voided at the end of two hours is determined by comparison in the colorimeter with a standard solution.

Reagents: Phenolsulphonephthalein solution in ampoules.
Standard solution of the same containing 0.006 gm. to the liter.
10% solution of sodium carbonate.

Technique: The patient drinks a glass of water and about half an hour later the bladder is emptied, either spontaneously or by catheter. Exactly 1 c.c. of the solution from an ampoule is then injected subcutaneously into some part of the trunk free from edema, preferably the lumbar region, using for the purpose an accurately graduated syringe. At the end of two hours from the time of injection the patient empties the bladder again or is catheterized and the urine is collected. In some cases a catheter is placed in position when the injection is given and the urine as

it trickles out is collected in a receptacle containing a few drops of 10% sodium carbonate solution. The time of the first development of a pink coloration of the fluid in the receptacle is noted, as this indicates the period elapsing before the first elimination of the dye. In acid urine the dye remains colorless, therefore the entire two hour amount of urine is first made strongly alkaline with sodium carbonate solution, causing it to become more or less intensely red according to the amount present. It is then diluted to a definite volume giving an intensity of color as nearly as possible like that of the standard and enough is filtered to fill the cup of the Hellige or Duboscq colorimeter. The former gives the percentage directly (which must be corrected for the dilution); with the latter the calculation is made as follows:

Result:

$$X = \frac{S}{R} \times \frac{Rd}{Sd} \times 100$$

X = percentage of phenolsulphonephthalein.
S = reading of standard.
R = reading of urine.
Rd = dilution of urine.
Sd = dilution of standard.

If the standard is set at 10 and the dilution of the standard is to 1000 the formula becomes $X = \frac{Rd}{R}$, that is, the percentage equals the dilution of the urine divided by the reading of the urine. In healthy persons the drug usually appears in the urine in from 5 to 10 minutes, and the total excretion at the end of two hours is from 50 to 85 per cent.

DIRECTIONS FOR COLLECTING BLOOD FOR ANALYSIS.

When the uric acid content of the blood is to be determined, the patient should be on a purin-free diet for at least two days before the sample is taken.

When the urea and non-protein nitrogen are to be determined, the patient should be on a low protein diet for at least two days before the sample is taken.

Blood samples are always to be taken before the patient has

breakfast. Blood in which the sugar is to be determined must be sent to the laboratory immediately after collection, as delay renders the results inaccurate.

The following amounts are necessary:

Urea and non-protein nitrogen.....15 c.c.
Uric acid.....20 c.c.
Sugar and creatinine.....10 c.c.
All the above in one specimen.....35 c.c.

A suction apparatus and graduated bottles for collecting the blood and a supply of powdered potassium oxalate will be furnished by the laboratory.

The rubber stopper with suction tubes attached is sterilized by boiling. According to the amount of blood to be drawn, place one or two knife points of the oxalate in the bottle, insert the stopper, and introduce the needle into the vein.

Aspirate the blood by suction on the mouth piece, shaking the bottle from time to time to prevent clotting. Stopper the bottle with a cork and shake *thoroughly*. Label as follows:

Name
Date
Examination required
Diagnosis
Ward
Hour taken

MCLEAN COEFFICIENT.

When this is to be determined, the following directions must be observed:

The patient is to be given 150 to 250 c.c. of water to insure a free flow of urine. One hour later the bladder is emptied, by catheter if necessary, and the time is noted to within one minute. About 36 minutes later 10 to 15 c.c. of blood is withdrawn and prevented from clotting by the use of potassium oxalate as usual. At the end of exactly 72 minutes from the time of voiding the bladder is again emptied and the entire specimen, taking care to avoid the least loss, is at once sent to the laboratory, together with the specimen of blood. The specimens must reach the laboratory before 10 A. M. The patient must take no food or drink during the 72 minute period. The patient's weight, taken on the day of the test, must be stated on the label of the blood specimen.

DETERMINATION OF TOTAL NON-PROTEIN NITROGEN IN BLOOD.

Folin and Denis.

Principle: The protein substances of the blood are precipitated by the action of an excess of methyl alcohol, complete clarification being secured by the addition of zinc chloride. The nitrogen in an aliquot part of the filtrate is converted into ammonia by digestion with concentrated sulphuric acid and the ammonia is then transferred to an acid solution by aeration. The amount of ammonia may be determined either by Nesslerization or by titration with N/100 alkali solution.

Reagents: Methyl alcohol (acetone free).
Saturated alcoholic solution of zinc chloride.
Potassium sulphate.
5% solution of copper sulphate.
Kerosene.
Saturated solution of sodium hydrate.
Caprylic alcohol.
Nessler's solution*.
Concentrated H_2SO_4.
N/100 H_2SO_4.
N/100 NaOH.
Alizarin.
Standard ammonium sulphate solution†.

Technique: Five c.c. of the blood obtained by puncture from a vein and kept from clotting by the use of a little finely powdered potassium oxalate (about 0.1 gm. for 20 c.c. of blood) is allowed

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*Nessler's solution is made as follows:

Mercuric iodide	200 gm.
Potassium iodide	100 gm.
Potassium hydroxide	400 gm.

Rub the red iodide to a smooth paste with water and transfer to a two liter flask. Grind the potassium iodide to a powder in the same mortar and add to the iodide in the flask using about 800 c.c. of water. Dissolve the potassium hydroxide in about one liter of water, cool thoroughly, and then add with constant shaking to the mixture in the flask. Make up to volume. The solution usually becomes perfectly clear. Place in incubator at 37 to 40 C. over night or until the yellowish-white precipitate which may settle out is thoroughly dissolved and only a small amount of dark brownish-red precipitate remains. The solution is then ready to be siphoned off and used.

†Standard ammonium sulphate solution. This solution, of which 5 c.c. contains 1 mgm. of nitrogen, is made by dissolving either 0.944 gm. of ammonium sulphate or 0.0764 gm. of ammonium chloride of highest purity in 1000 c.c. of distilled water.

to flow into a 50 c.c. volumetric flask half filled with acetone-free methyl alcohol. The flask is filled to the mark with methyl alcohol and is well shaken. At the end of two hours the fluid is filtered and the filtrate containing the non-protein nitrogenous constituents is further clarified by the addition of a few drops of saturated alcoholic solution of zinc chloride and re-filtration at the expiration of 5 to 10 minutes. The filtrate should now be quite colorless. Ten c.c. of this (equivalent to 1 c.c. of blood) is pipetted into a Jena glass test tube about 200 x 20 mm. and 0.5 grams of potassium sulphate, two drops of 5% copper sulphate solution, one c.c. of concentrated sulphuric acid and three drops of kerosene are added. The mixture is heated in a water bath until the alcohol has been driven off and then over a micro-burner until digestion is complete, as shown by the mixture becoming perfectly clear. It is boiled for two minutes longer to insure complete breaking down of the organic compounds, is allowed to cool, and is then carefully diluted with about six c.c. of water. The ammonia is removed from this fluid by aeration and the addition of alkali. For this purpose a receiving cylinder is arranged containing 15 c.c. of water, and two drops of 10% hydrochloric acid. The test tube containing the digestion mixture is placed in the other cylinder of the aeration apparatus and three c.c. of saturated sodium hydroxide solution is added. According to the strength of the air current aeration is complete in from fifteen minutes to half an hour. The ammonia in the acid solution may be determined either by Nesslerization or by titration.

A. Nesslerization. Into a 100 c.c. cylinder put 5 c.c. of standard solution and 50 c.c. of distilled water. Dilute 10 c.c. of Nessler's solution with 50 c.c. of water and add 25 c.c. of this mixture to the standard, then making up the volume to 100 c.c. with water. Without delay add 8 to 10 c.c. of the diluted Nessler's solution to the unknown solution in a 100 c.c. cylinder and at once dilute to 25, 50 or 100 c.c. according to the depth of color produced, using the dilution which comes nearest in depth to that of the standard. The standard solution is placed in one cup of the Duboscq colorimeter and the unknown is compared with it.

Result: $X = S \times \frac{Rd}{R}$

X = mg. of non-protein nitrogen per 100 c.c. of blood.

S = reading of standard.
R = reading of unknown.
Rd = volume in c.c. to which the unknown is diluted.

B. If the ammonia is to be determined by titration the receiving cylinder of the aeration apparatus must contain 20 c.c. of N/100 hydrochloric acid. The amount of this left un-neutralized by the ammonia formed in the blood solution is then determined by titration with N/100 sodium hydrate solution, using alizarin as indicator.

Result: Each c.c. of N/100 ammonia is equivalent to 0.00014 gm. of nitrogen.
The non-protein nitrogen in health is usually from 25 to 30 mgm. per 100 c.c. of blood.

DETERMINATION OF UREA IN BLOOD

VAN SLYKE AND CULLEN.

Principle: Urease, an enzyme obtained from the soy bean, converts urea into ammonium carbonate. It acts at room temperature, but is most active at a temperature of about 55 C. and when in a perfectly neutral solution. It decomposes nothing but urea, and is not interfered with by the presence of any other substance occurring in the blood. The urea in the blood is converted into ammonium carbonate by the enzyme, the ammonia in the latter is then liberated by the addition of alkali, and is aspirated into a measured amount of standard acid. The ammonium carbonate formed would by its alkaline reaction retard the action of the enzyme, but the development of alkalinity is prevented by the presence of potassium acid phosphate (KH_2PO_4). An excess of this, however, interferes with the combination of enzyme and urea which precedes hydrolysis of the latter so that it is advisable to use the minimum amount of phosphate that will keep the reaction sufficiently near the neutral point.

Reagents: Soy bean enzyme (Urease)*
N/100 H_2SO_4
N/100 NaOH
0.6% solution of acid potassium phosphate, (KH_2PO_4)

*See determination of urea in urine.

Alizarin
Nessler's solution
Saturated solution of potassium carbonate. (90 grams to 100 c.c. water)
Standard solution of ammonium sulphate (1 mgm. of nitrogen to 5 c.c.)
Caprylic alcohol

Technique: Into a large test tube introduce 3 c.c. of 0.6% KH_2PO_4 solution, and 1 c.c. of 10% soy bean urease solution. Add 2 c.c. of blood drawn from a vein and prevented from coagulation by the addition of potassium oxalate (see non-protein nitrogen determination). Add 4 to 5 drops of caprylic alcohol to prevent foaming and allow to stand for 15 minutes. Then arrange the aeration apparatus as for non-protein nitrogen determination, add an equal volume of saturated potassium carbonate solution to the mixture and aerate for 20 minutes to one half hour. The nitrogen is determined by Nesslerization or by titration, as described under non-protein nitrogen.

Result: Nesslerization.

$$X = \frac{S}{R} \times \frac{Rd}{2}$$

X = mgm. of urea nitrogen per 100 c.c. of blood.
S = reading of standard.
R = reading of unknown.
Rd = dilution of unknown.

If the result is to be determined by titration place in the receiving cylinder 15 c.c. of N/100 acid and titrate back with N/100 alkali. Each c.c. of acid neutralized by the ammonia formed is equivalent to 0.00014 gm. N or 0.3 mg. of urea. The amount of urea may also be determined by multiplying the urea nitrogen by the factor 2.14.

Theoretically a second determination should be done without the use of urease in order to determine the ammonia alone in the blood so that this may be subtracted from the amount obtained from the tube containing the solution treated with urease. This fraction is however extremely small and ordinarily may be neglected.

The urea N of the blood in health is ordinarily about 11 to 15 mgm. per 100 c.c.

DETERMINATION OF URIC ACID IN BLOOD.

FOLIN AND DENIS.

Principle: The protein matter is removed from the blood and the uric acid is precipitated from the resulting clear solution by the addition of ammoniacal silver solution. This precipitate is re-dissolved with potassium cyanide and the resulting solution is treated with phosphotungstate reagent which gives a blue color proportional in depth to the amount of uric acid present. This is compared in the colorimeter with the color produced by a standard solution of uric acid under similar conditions (see determination of uric acid in urine.)

Reagents: N/100 acetic acid.
Alumina cream.*
Ammoniacal silver magnesia solution.
5% potassium cyanide solution.
Uric acid reagent.
20% sodium carbonate solution.
Uric acid standard solution.

Technique: To 100 c.c. of boiling N/100 acetic acid in a casserole add slowly 15 to 20 c.c. of blood carefully measured, and bring to the boiling point. Add 5 to 8 c.c. of alumina cream and continue boiling until the precipitate separates completely and the fluid when examined by letting it drop from a stirring rod is water clear. Filter through a hardened filter, wash the precipitate back into the casserole, wash it with boiling water, and filter again. The filtrates, which should be perfectly clear, are concentrated on a water bath to a low volume, are united, and then brought down to a final volume of about 2 c.c. If any protein precipitates, it should be removed by centrifuging and washing the sediment. The fluid and washings, which should not exceed 10 c.c., are transferred quantitatively to a centrifuge tube and treated with 20 drops of ammoniacal silver magnesia solution. Mix thoroughly, allow to stand a few minutes in a cold place (refrigerator) and centrifuge. Pour off the supernatant fluid completely, inverting the tube over a piece of filter paper and

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*Alumina cream is made as follows:
5% sodium bicarbonate solution.....100
4% aluminum acetate solution.....200
Allow the aluminum hydroxide to settle and decant three times with distilled water. Preserve in the form of a fairly thick magma.

removing ammonia vapor by introducing into the mouth of the tube a rubber tube connected with a suction pump. Add one or two drops of 5% potassium cyanide solution, stir well, and then add a few drops of water and stir again. Add one or two c.c. of uric acid reagent according to the bulk of the precipitate and either 5 or 10 c.c. of 20% sodium carbonate solution as the case may be. In a 100 c.c. cylinder place 5 c.c. of the uric acid standard solution, add the same number of drops of potassium cyanide solution as were used for the unknown, 2 c.c. of uric acid reagent and 10 c.c. of 20% sodium carbonate solution. At the end of about half a minute dilute to 100 c.c. Transfer the blue unknown solution quantitatively to a 50 c.c. cylinder and dilute to 25 or 50 c.c. according to its depth of color. Compare with the standard in the Duboscq colorimeter.

Result: $X = \frac{S}{R} \times \frac{V}{W}$

X = mgm. of uric acid per 100 c.c. of blood.
S = reading of standard.
R = reading of unknown.
V = volume to which unknown was diluted.
W = volume of blood used in analysis.

The blood in health usually contains 1 to 2 mgm. of uric acid per 100 c.c.

DETERMINATION OF SUGAR IN BLOOD.

LEWIS AND BENEDICT.

Principle: The blood is freed from protein by the use of picric acid. The glucose in the resulting filtrate when heated in the presence of picric acid and alkali gives rise to a reddish-brown color proportional in intensity to the amount of glucose present. By comparison with a standard solution in the Duboscq colorimeter the amount of sugar is determined.

Reagents: Picric acid.
10% sodium carbonate solution.
Picramic acid solution.*

*The picramic acid solution is made as follows:

Picramic acid	60 mg.
Sodium carbonate anhydrous	100 mg.
Distilled water to make	1000 c.c.

The solution should be standardized before use so that it gives the

Technique: Two c.c. of blood, which has been prevented from clotting by the use of potassium oxalate is measured into a test tube with an Ostwald pipette. The pipette is rinsed with water and the amount made up to 10 c.c. After shaking thoroughly in order to lake the corpuscles about 0.2 gm. of picric acid is added to precipitate the protein and saturate the solution with the acid. Allow the mixture to stand for several minutes and then centrifuge. Filter the clear supernatant liquid through a small paper into a test tube and measure three c.c. of the filtrate into a test tube provided with marks at 10, 15, and 20 c.c. Add one c.c. of 10% sodium carbonate solution and heat in a water bath for 15 minutes to cause the color produced by the alkali, picric acid, and glucose to reach its full intensity. Allow the solution to cool and with distilled water dilute to 10, 15, or 20 c.c. in order to produce a color as nearly as possible like that of the standard. The blood solution is compared with the standard at once to avoid color changes and the result is calculated as follows:

Result:

$$X = \frac{S}{R} \times \frac{m}{W} \times 100$$

X = mgm. of dextrose per 100 c.c. of blood.
S = reading of standard.
R = reading of unknown.
m = mgm. of dextrose to which the standard corresponds.
W = number of c.c. of blood used in the determination.

W and m are each equal to 0.6 and therefore cancel, so that

$$X = \frac{S}{R} \times 100.$$

In health, the blood sugar is usually between 75 and 120 mgm. per 100 c.c.

DETERMINATION OF CREATININE IN BLOOD.
MYERS AND FINE.

Principle: The proteins are precipitated by the use of picric acid. The creatinine present gives a brownish color on the addi-

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same color in the colorimeter as a solution containing 0.6 mgm. of glucose treated with picric acid and sodium carbonate as described above and made up to 10 c.c.

tion of alkali and the amount can be determined by comparison with a standard solution in the Duboscq colorimeter

Reagents: Picric acid.
10% sodium hydroxide solution.
Standard creatinine solution.*

Technique: Six c.c. of blood prevented from clotting by the use of potassium oxalate is diluted to 30 c.c. with distilled water in a large centrifuge tube. After the corpuscles have been laked one half to one gram of picric acid is added and the mixture is stirred thoroughly. After complete precipitation of the protein substances has taken place the mixture is centrifuged and the supernatant fluid is filtered off. Ten c.c. of the filtrate (equivalent to 2 c.c. of blood) are measured into a large test tube and one-half c.c. of 10% sodium hydroxide is added. To 10 c.c. of the standard creatinine solution add the same amount of alkali and compare the two in the Duboscq colorimeter. Enough filtrate will have been obtained for a sugar determination on the same specimen.

Result:

$$X = \frac{S}{R} \times \frac{Rd}{Sd} \times \frac{100}{W} \times \frac{1}{2}$$

X = mgm. of creatinine per 100 c.c. of blood.
S = reading of standard.
R = reading of unknown.
Rd = dilution of unknown.
Sd = dilution of standard.
W = amount of blood in c.c. taken for determination.

The amount of creatinine present in health is usually from one to two mgm. per 100 c.c. of blood.

1 mgm. of creatinine is equivalent to 0.0371 mgm. of N.

DETERMINATION OF CREATINE IN BLOOD

Principle: On heating creatine to 120 C. in the presence of acid it is dehydrated, and its anhydride, creatinine, is formed.

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*This solution is made as follows:

Dissolve an accurately weighed amount of creatinine in N/10 HCl (about 1 mgm. to 1 c.c.) and dilute this solution with saturated picric acid solution so that the finished product contains exactly 5 mgm. of creatinine per 100 c.c.

Reagents: Picric acid.
10% sodium hydroxide solution.
Standard creatinine solution.

Technique: Place 5 c.c. of the filtrate obtained as for the creatinine determination in a test tube and heat in the autoclave at 15 to 20 pounds pressure (1000 to 1250 gm. per square cm.) for 20 minutes. Cool, dilute to 10 c.c. and then proceed as described for creatinine determination.

Result: From the amount of creatinine so obtained deduct that from the unheated blood. The difference will be the creatine content of the blood in terms of creatinine. To obtain the amount of creatine multiply this figure by the factor 1.16. The amount of creatine in health is usually 5 to 10 mg. per 100 c.c. of blood.

DETERMINATION OF CHLORIDES IN BLOOD.

MCLEAN AND VAN SLYKE.

Principle: The proteins are removed from the blood plasma by coagulation by heat in acid solution in the presence of an excess of magnesium sulphate. The fluid is clarified by the addition of animal charcoal and in the clear filtrate the chlorides are determined by titration with potassium iodide. For this purpose an excess of standard silver nitrate solution is added, the resulting precipitate of silver chloride is removed by filtration, and the amount of silver remaining uncombined is determined by titration with potassium iodide in the presence of nitrous acid and starch. As soon as the iodine has combined with all the silver, any additional amount added gives a blue color with the starch.

Reagents: 10% magnesium sulphate solution
Blood charcoal (Merck's Reagent; chloride free)
Silver nitrate solution (1 c.c. equivalent to 2 mgm. of NaCl.)

Silver nitrate	5.812	gm.
Nitric acid (sp. gr. 1.42)	250.	c.c.
Water to	1000.	c.c.

Potassium iodide solution (1 c.c. equivalent to 1 mgm. of NaCl.)

Potassium iodide	3.0	gm.
Water to	1000.	c.c.

Starch solution

Sodium citrate	($Na_3C_6H_5O_7 + 5½ H_2O$) 446.	gm.
Sodium nitrite	20.	gm.
Soluble starch	2.5	gm.
Water to	1000.	c.c.

Dissolve the starch in 500 c.c. of warm water, add the salts and heat till dissolved. While still hot filter through cotton, wash the filter with hot water, and after cooling, make the filtrate up to 1000 c.c. The solution becomes cloudy on standing, but keeps indefinitely.

The citrate and nitrite are present in order to secure the optimum conditions of acidity and to liberate the iodine from the iodide.

The potassium iodide solution is standardized against the silver solution by adding 5 c.c. of the starch solution to 5 c.c. of the silver solution and titrating with the iodide solution to the first appearance of a blue color. The iodide solution is then diluted so that 10 c.c. are exactly equivalent to 5 c.c. of the silver solution.

Technique: Centrifuge 5 c.c. or more of oxalated blood (see determination of non-protein nitrogen), and with an Ostwald pipette measure 2 c.c. of the plasma so obtained into a 20 c.c. stoppered volumetric flask containing 10 c.c. of 10 per cent. magnesium sulphate solution, rinsing the pipette twice by drawing up the solution. Add two drops of 50 per cent. acetic acid, add water to the 20 c.c. mark, mix, stopper lightly, and heat in the water or steam bath to 100 C. for ten minutes. Allow to cool and mix with about 0.3 gm. of blood charcoal (Merck's Reagent) in a beaker. Filter through a dry folded filter until a water clear filtrate is obtained. Measure 10 c.c. of the filtrate into a 25 c.c. volumetric flask, add 5 c.c. of the silver nitrate solution (equivalent to 10 mg. of NaCl), and make up to 25 c.c. Add two drops of caprylic alcohol, stopper the flask, and invert several times. Allow to stand five minutes, and filter through a dry folded filter, obtaining a clear colorless filtrate. To 20 c.c. of the filtrate add an amount of the starch solution equal to the amount of silver solution present in the volume of filtrate used, e.g., in the

present instance (5 c.c. of silver solution diluted to 25 c.c. and 20 c.c. of filtrate taken) 4 c.c. of silver solution is present and 4 c.c. of starch solution must be added. Then titrate with the potassium iodide solution to the appearance of the first definite blue color.

Result: Using 20 c.c. of filtrate from the silver chloride,

$$X = \frac{12.5\ (8 - Y)}{F}$$

X = gm. of NaCl per liter
Y = c.c. of KI solution used in titration
F = c.c. of blood filtrate taken

The chlorides in human plasma are normally from 5.62 to 6.25 gm. of sodium chloride per liter, according to the amount ingested.

FAT DETERMINATION IN FECES.

Principle: The soaps present in the stool, which are insoluble in ether, are split by the addition of acid alcohol into free fatty acids and neutral salts. The dried material is then extracted in the Soxhlet apparatus, the result giving the total of the ether soluble bodies in the stool and including small amounts of cholesterin, lecithin, and coprosterin, etc.

Reagents: 1% hydrochloric acid in alcohol.
Ether.
Anhydrous ether.

Technique: A small quantity of acid alcohol is added to the dried and pulverized fecal material and thoroughly mixed. The mass is evaporated to dryness on the water bath and about 5 gm. of the resulting powder is placed in a paper extraction capsule and extracted with ether in the Soxhlet apparatus for 24 to 72 hours. The extract is evaporated to dryness and the residue is dissolved in anhydrous ether and filtered into a weighing bottle, the filter being washed with a little fresh ether which is added to the filtrate. The entire filtrate is then evaporated to dryness and the weight determined.

Result: In adults the daily loss of fat in the stools may be put at 5 gm., or from 15 to 30% of the dried residue, but these figures are subject to great modification according to the diet.

DETERMINATION OF FAT IN MILK.

BABCOCK.

Principle: By the action of strong sulphuric acid the protein of the milk is destroyed and the fat may easily be centrifugalized from the hot fluid. A mixture of amyl alcohol and hydrochloric acid is added to facilitate the separation of the fat.

Reagents: Babcock sulphuric acid (Sp. Gr. 1.825)*
Amyl alcohol mixture.†

Technique: *Thoroughly* mix the sample of milk. With the special pipette measure 17.6 c.c. (the average volume of 18 gm.) into each of two Babcock bottles of equal weight and add a similar volume of the Babcock sulphuric acid. Mix carefully by shaking the bottle with a rotary motion. Centrifuge immediately for about four minutes and add two c.c. of the amyl alcohol mixture and enough of a hot freshly made mixture of equal parts of concentrated sulphuric acid and water to bring the fluid to the neck of the bottle. Centrifuge again for two minutes, and then again add enough of the hot sulphuric acid and water mixture to bring the level of the fluid nearly to the top of the graduation in the neck. Make the reading with dividers from the top of the upper meniscus to the bottom of the lower one. For breast milk use the special small tubes requiring only 5 c.c. of milk. The average composition of milk is as follows:

	Human	Cow's
Fat	4.00	3.50
Sugar	7.00	4.30
Protein	1.50	4.00
Ash	0.20	0.70
Water	87.30	87.50

Total solids in each case are about 12.50.

COLLOIDAL GOLD REACTION

Principle: If a cerebrospinal fluid is diluted in geometrical progression with a weak neutral electrolyte and five volumes of clinical colloidal gold are added to each successive solution, a

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*Concentrated sulphuric acid, 96%, Sp. gr. 1.84.....1800 c.c.
Distilled water ..... 189 c.c.
N. B. Add acid to water, not vice versa.
†Amyl alcohol.
Concentrated hydrochloric acid: equal parts.

series of precipitation colors may be obtained which, when plotted as abscissæ against the dilutions as ordinates, will give a curve whose contour is characteristic for certain of the infectious diseases of the central nervous system and meninges.

Reagents: Clinical colloidal gold.
0.4% solution of sodium chloride.

Technique:

THE PREPARATION OF CLINICAL COLLOIDAL GOLD. (O. I. LEE.)

Reagents: 1% gold chloride acid solution
2% potassium carbonate solution
1% oxalic acid solution
2.5% solution of formalin containing approximately 37% of formaldehyde (5 c.c. of formalin plus 200 c.c. of distilled water).

The solutions should be made up just before use from fresh distilled water. The gold chloride of Merck and Company in sealed ampoules is recommended. Any high grade will be found satisfactory for the other chemicals.*

To 1 liter of distilled water in a narrow neck glass-stoppered flask free from scratches and cleaned by treatment with warm aqua regia† and subsequent thorough rinsing, add 10 c.c. of the 1 per cent. gold chloride, 7 c.c. of the 2 per cent. potassium car-

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* In order that the correct amount of formaldehyde should be used, it is necessary to determine the value of the formalin.

Evaluation of Formalin.
Modified Method of Ronijn.

Reagents: N/10 iodine solution (12.692 gm. of resublimed iodine dissolved in 150 c.c. of 10% potassium iodide and made up to 1 liter.)
2 N sodium hydroxide solution.
N/10 sodium thiosulphate (24.85 gm. of pure crystallized $Na_2S_2O_3\ 5H_2O$ made up to 1 liter)
HCl concentrated.

Ten c.c. of the formalin is made up to 1 liter with distilled water and 5 c.c. of this solution is mixed with 25 c.c. of the decinormal iodine. Two N sodium hydroxide is added, drop by drop, until the liquid becomes clear yellow. After ten minutes, 2 c.c. of concentrated hydrochloric acid are added and the free iodine titrated with decinormal sodium thiosulphate. Two atoms of iodine are equivalent to one molecule of formaldehyde. If T equals the titration, and S the specific gravity of the formalin, then

$$\text{Per cent. of } CH_2O \text{ equals } \frac{75 - 3\ T}{S}$$

† Seven volumes of concentrated hydrochloric acid to two volumes of concentrated nitric acid.

bonate, 1.75 c.c. of 1 per cent. oxalic acid, and 0.83 c.c. of 2.5% formalin (37% CH_2O), or equivalent amount of 8.07 mg. of CH_2O per liter of colloidal gold made. An Ostwald pipette is used for measuring the last reagent. Mix thoroughly, and heat over a Bunsen burner to between 80 and 85 C. Then reduce the flame enough to keep the temperature constant. The thermometer used must have had all the filler removed from the etched markings. The wire gauze supporting the flask should be covered with a sheet of asbestos cardboard to prevent local reduction on that portion of the flask directly over the flame.

The solution will slowly develop a series of colors substantially as given below, though some of the shades are much more transient than others. The associated temperatures are merely typical and were recorded for a development lasting about one hour. The color changes come gradually in good colloidal gold solutions.

Color	Temperature Degrees C
Faint blue green	65
Pale blue green	74
Pale bluish lavender	78
Bluish lavender	79
Pinkish lavender	80
Bluish pink	82
Pale pink	83
Pink	84
Deep pink	84
Violet red	84
Bluish red	83
Deep bluish red	83
Deep ruby red	83
Dark ruby red (violet red in test tube or pipette)	83
Rebound or color transition	
Orange red (orange pink in test tube or pipette)	83

When the solution reaches its maximum depth of color, a remarkable lightening and change of color occurs within the space of a few seconds, and *until this rebound and transition of color has taken place the solution will be worthless for clinical*

purposes. After this point has been passed, the solution is allowed to cool spontaneously to room temperature.

If a solution is heavily clouded, pour it out and examine the flask for spots and streaks of reduced gold transmitting green, blue or violet light. If noticeably present, treat with warm aqua regia for a few minutes, rinse thoroughly, and repeat the experiment. A clear colloidal gold solution leaves an unspotted container.

If the solutions still continue to be turbid and show a strong brownish fluorescence, a series of half a dozen liters should be made with amounts of 2.5 per cent. formalin, decreasing and increasing by 0.05 c.c. from that originally used and notice taken in which direction improvement occurs.

A slight brownish dispersion is no bar to the usefulness of the colloidal gold, but a six inch layer should be transparent, and 5 c.c. should be completely precipitated in one hour by 1.7 c.c. of 1 per cent. sodium chloride.

The correct color of the finished solution is matched almost exactly in a test tube comparison by a solution of 5 c.c. of 20 per cent. by weight of cobaltous nitrate ($Co(NO_3)_2.6\ H_2O$ plus 0.30 c.c. of 0.25 per cent. of potassium dichromate ($K_2Cr_2O_7$) solution, plus 10 c.c. of water. If the solution falls away in the direction of the blue from this standard, it should be rejected.

The solution should be stored in the dark in a container of Pyrex or Jena glass cleaned with aqua regia.

Technique of Test: Into the first of a series of eleven heavy glass lipless test-tubes, measuring 11⁄16 x 6 inches, 1.8 c.c. of the 0.4 per cent. NaCl is measured and into each of the remaining ten, 1 c.c. Of the spinal fluid to be tested (clear and free of erythrocytes, sediment, or moulds), 0.2 c.c. is then introduced into the first tube with a 1 c.c. Ostwald pipette graduated to the tip and provided with a wide margin between the 1 c.c. mark and the top. One c.c. of the mixture is placed into the second tube, 1 c.c. of this mixture into the third, and so on up to and including the tenth tube, from which, however, the 1 c.c. withdrawn is rejected, leaving the eleventh tube as a color control. The concentration of the spinal fluid thus ranges from 1:10 in the first tube to 1:5120 in the tenth. To each of the eleven tubes is then added 5 c.c. of the colloidal gold solution measured with a short pipette, and the contents mixed by a rotary motion.

At the end of half an hour the color of each of the ten tubes is matched against a diffuse white background, with the nearest shade selected from the following color scale, using the eleventh tube for comparison.

Color	Numerical Color Value
Colorless	7
Pale blue	6
Steel blue	5
Blue	4
Blue violet, Violet blue	3
Violet (Blue red, Red blue)	2
Violet red, Red violet	1
Red	0

The corresponding numerical color values (abscissæ) are then plotted on coordinate paper against the dilutions (ordinates), multiplying the ordinate values by some convenient factor (5/3), to elongate the curve formed by connecting the points in a continuous line. This constitutes the preliminary curve.

At the end of twenty-four hours the color values are again taken, and the curve obtained designated as final.

Notes on Interpretation of Curve

A normal spinal fluid always gives a straight line (0 0 0 0 0 0 0 0 0 0), but a straight line curve does not necessarily indicate that the patient's spinal cord is normal; for instance, myelitis has given 0 0 0 0 0 0 0 0 0 0. General paresis (final) usually gives complete precipitation in the first five, six, or seven tubes, followed by gradations towards the red with the higher dilutions unchanged, as 7 7 7 7 7 6 3 2 1 0. Often, however, there may be a reversion of color causing a characteristic step-ladder or terraced curve (7 6 1 3 3 3 2 0 0 0).

Tabes and lues are very similar, but speaking generally, the luetic curve is inclined to be narrower, more to the left, and not so high as that of tabes. Thus, lues may be 2 2 3 2 2 2 1 1 0 0, and tabes 2 2 3 4 3 2 1 0 0 0.

In meningitis, reduction (peak of curve) is almost always to the right of the center, or on the extreme right.

Example: pneumococcus meningitis 0 1 1 1 1 1 3 4 4 3.

Except in general paresis, values exceeding 4 are not likely to be obtained, and the preliminary and final curve will be found coincident or roughly parellel. In paresis, however, the first half of the preliminary curve often lags progressively behind the first half of the final curve. Preliminary 2 3 4 5 4 2 1 0 0 0; final 7 7 7 7 7 2 1 0 0 0.

DETECTION OF MERCURY IN EXCRETIONS.

Principle: The organic combination in which the mercury exists is broken down by oxidation with nascent chlorine. By electrolytic double decomposition, the metal is then caused to become deposited on a piece of copper wire and from this it is distilled on to a piece of dentists' gold foil. The amalgam so formed is recognizable as a silvery patch of discoloration on the gold. In collecting the specimens and in carrying out the tests it is necessary to use only receptacles and apparatus that are chemically clean.

Reagents: Potassium chlorate
Concentrated HCl
Bare copper wire (B & S No. 14 is a convenient size)
Soft glass tubes, 2 mm. inside diameter, 10 cm. long, sealed at one end.
Small pellets of gold foil as used by dentists.*
Microburner.

Technique: As large a volume as possible of the material (urine, gastric lavage fluid, colon irrigation) is acidulated with 10 to 20 c.c. of concentrated hydrochloric acid; a few grams of potassium chlorate are added; and the whole is heated in a large porcelain evaporating dish. It is not advisable to use more acid or chlorate than is required for complete decolorization, the exact amounts being readily judged by experience. Stools, vomitus, blood, etc., are first diluted with several volumes of water, and require more of the oxidizing materials. The excess of acid and chlorine is eliminated by evaporation, and the solution concentrated to about 25 c.c. Any solid matter, especially fat, is filtered off; and into the filtrate, contained in an Erlenmeyer flask, is

*Manufactured by J. M. Ney Co., Hartford, Conn. Sold by dentists' supply firms in 1/16 oz. vials under the trade designation No. 1/4 velvet cohesive gold cylinders.

dropped a straight piece of the copper wire, 2 cm. long, previously cleaned by a short immersion in concentrated nitric acid followed by washing with distilled water. It is allowed to remain in the fluid for several hours or over night, preferably in a warm place. In an emergency the time may be shortened by boiling the fluid with the wire for five minutes as a preliminary test, but if the result is negative the test must be repeated in the regular way. The wire is washed with distilled water by decantation, dried by rolling very gently on a filter paper, and slipped to the bottom of the glass tube, avoiding abrasion. It is then followed by a cylinder of gold foil pushed to within 2 cm. of the wire.

Holding the tube horizontally, its closed end is carefully heated in the flame of the microburner almost to the softening point, after which the tube is gently warmed close to the gold cylinder, the edges of which should be frequently examined during this operation for any trace of a silvery discoloration signifying the presence of mercury. A hand lens is useful in recognizing very small amounts of the metal. If chlorine is still present in the concentrated oxidized solution the wire may be completely dissolved, in which case the solution should be diluted, again concentrated by boiling, and another wire dropped in. If the gold is too close to the wire, or the tube is heated too strongly, the mercury may be driven into or beyond the gold, the discolorization then being transient.

By this method it is possible to recognize the presence of 0.01 mg. of mercury in the amount of material taken for analysis. Calomel in therapeutic doses occasionally gives a positive reaction in the urine.

BACTERIOLOGY

GENERAL INSTRUCTIONS FOR LABORATORY WORKERS

The worker in the bacteriological laboratory of a general hospital is constantly handling infectious materials. Either through indifference or through lack of knowledge he may subject himself to serious consequences, as well as be the source of great danger to others. In order, therefore, to protect the worker and others against the possibility of infection, the following rules have been adopted and must be fully observed:

1. While in the laboratory, wear the regulation laboratory gown, which entirely covers the clothing. If infectious material is spilled on the gown, remove it and sterilize it in the autoclave.

2. Do not eat or drink in the bacteriological laboratory, nor place a pencil or other object in the mouth.

3. Moisten labels with water, not with the tongue.

4. Cleanse hands in disinfecting solution in basin, then wash thoroughly with soap and running water, before leaving the laboratory for meals or at end of work.

5. Do not leave infectious materials openly exposed.

6. Throw no waste materials on floor; place them in the pails or jars provided. Never throw infected materials into these receptacles.

7. Do not place stoppers removed from tubes or flasks containing live cultures on work table or other object, and always return stopper to tube or flask before discarding.

8. After finishing with tubes, flasks, or plates containing live cultures, place them in the small boiler which is provided at each work table. Keep the boiler covered when not in use.

9. Pour no infectious material into the sink or drain.

10. If infectious material is spilled on work table or floor flood with 5 per cent. carbolic acid, and allow to remain at least

one hour. Boil or place in the autoclave other objects accidentally infected.

11. If the hands become accidentally contaminated with infectious material or after such material has been handled, at once disinfect by washing for some time in a 1:1,000 solution of bichloride of mercury, which is found in basin placed on the work table, then scrub with soap and running water. Do not wipe hands on towel until they are thoroughly disinfected.

12. In handling infectious material use only pipettes plugged with cotton. After using, sterilize by boiling or by submerging completely in 2.5 per cent. cresol solution in the cylinder which is placed on each work table.

13. After taking blood cultures from patients with infectious disease, disinfect syringes and needles either by boiling or by submerging for some hours in 2.5 per cent. cresol solution. Place the other apparatus used in making cultures, such as agar tubes, etc., in small boiler to be sterilized.

14. Place animals inoculated with infectious material in proper isolation cages in the animal room. These cages must be cleaned and disinfected after use.

15. Take precautions with the body of an animal dead from inoculation, to prevent the spread of infectious material.

16. Nail animal to be examined post-mortem on wooden board; place in large enamel-ware tray; and wash the skin thoroughly with disinfecting solution.

17. Immediately after use, place in the instrument sterilizer all instruments used in the autopsy of an animal, and sterilize by boiling for 20 to 30 minutes.

18. Immediately after making an autopsy cover the body of the animal, without removing it from the board, with a thin layer of absorbent cotton thoroughly wet with disinfecting solution. Then remove to basement and place animal in covered metal can to be cremated. Sterilize the tray by flooding the bottom with 2.5 per cent. cresol solution and allowing it to stand for a few hours.

19. Report at once to the bacteriologist in charge all accidents, such as spilling of infectious materials, or any injury, such as cutting or pricking of fingers, burns, etc.

20. Permission must be obtained from the head of the department before any culture is taken out of the laboratory.

USE OF STERILIZERS.

Arnold Sterilizer. This is used in the sterilization of media containing substances (sugar, etc.) which are decomposed by heating at higher temperatures.

Always add water to the depth of 7 to 10 cm. (about 3 to 4 in.) to supply-pan before starting gas flame. Do not place tubes or flasks in the chamber until steam flows rapidly into it. Count time from moment when the materials are placed in the sterilizer. Heat fluid media in tubes 20 minutes in flowing steam, in large flasks 30 minutes; agar in tubes 25 minutes, in large flasks 30 to 45 minutes. When the time period is up, open door and at once remove the tubes or flasks. Repeat the heatings on three consecutive days to sterilize media.

Autoclave. If the ordinary upright laboratory autoclave with gas heater is used, always see that at least 10 cm. (about 4 in.) of water is in the bottom before starting the flame. After adding sufficient water, put in objects to be sterilized, then screw the lid down evenly all around and open the valve in the middle of the lid; then start flame. Leave valve open until steam flows rapidly from vent. Do not leave valve open long, as the autoclave will boil dry. Close valve and fasten clamps until no steam escapes from edge of lid. Allow pressure to rise until the gauge registers 15 lbs.; then lower flame, and maintain at this point for 30 minutes. Shut off gas flame, allow to stand until *pressure gauge returns to zero,* then open valve, and unscrew lid.

In case a more complicated sterilizer, like that for sterilization of surgical dressings, etc., is used, follow the directions which are furnished with the machine.

Dry Heat Sterilizer. The dry heat method of sterilization is used for glassware, glass syringes, needles, instruments, etc. Never use it for anything which is moist, only for dry objects. Since this apparatus is not under the control of an automatic regulator, the temperature must be regulated through the size of the gas flame, therefore, watch carefully while in use. Place in chamber the articles for sterilization; close the door; turn on and light the gas. Allow a full flame until temperature rises to 165 to 170 C., then lower the flame and maintain that temperature

for one hour. Higher temperatures will char cotton, and must, therefore, be guarded against. When the time limit is up, turn off the flame, and allow the temperature to fall below 100 C. before opening the chamber.

PREPARATION OF NUTRIENT MEDIA.

GENERAL DIRECTIONS

THE TITRATION AND ADJUSTMENT OF REACTION OF MEDIA.

I. Apparatus required: Two or three 100 c.c. porcelain casseroles or 100 c.c. Erlenmeyer flasks; two 50 c.c. burettes, graduated in 0.10 to 0.05 c.c., with suitable holder for operation; one 1 c.c. pipette, graduated in 0.10 c.c. or less; two or three 5 c.c. pipettes, graduated in 0.10 c.c.; one Bunsen burner; one iron tripod, height 7 inches, outside diameter 5 inches, with asbestos-filled wire gauze; glass stirring rods.

II. Reagents required: Indicator, 0.5 per cent. phenolphthalein dissolved in 50 per cent. alcohol; volumetric solutions, N/20 NaOH, N/1 NaOH, and N/20 HCl.

III. Procedure: First, see that all of the apparatus is thoroughly cleansed, and that the measuring burettes and pipettes are also dried before receiving the reagents, etc. Fill one of the burettes with N/20 NaOH solution and the other with N/20 HCl solution. Place 45 c.c. of distilled water in either a 100 c.c. white porcelain casserole or a 100 c.c. Erlenmeyer flask. To this add 5 c.c. of the medium, measuring most accurately with a 5 c.c. graduated pipette. In the case of a medium containing gelatin or agar, heat the distilled water before adding the sample. Add 1 c.c. of the phenolphthalein indicator; mix thoroughly with stirring rod; place container on wire gauze over Bunsen burner flame; and boil mixture for two minutes. Since most culture media require relatively more alkali at the boiling point than at lower temperatures to show the "end reaction", the titration should be carried out at that temperature as a constant. In general, most media are acid; therefore, only the N/20 NaOH solution is required; add this drop by drop from the burette, while constantly stirring or shaking the test sample. The "end reaction" is indicated by a faint but distinct pink color, which is uniform throughout the solution and permanent. Again heat

the sample to the boiling point for ten to fifteen seconds, and, if the color disappears, add immediately more of the N/20 NaOH solution to the same end reaction. Take this as the final reading for corrections of the reaction of the medium. It is desirable to titrate two or more samples from each lot of medium, and take the average in adjusting its reaction. The acidity or alkalinity of a medium is expressed in terms of that quantity of a normal (N/1) volumetric solution which would bring 100 c.c. to the neutral point, using phenolphthalein as an indicator. If acid, the plus sign (+) is placed before the number expressing this amount, if alkaline, the minus sign (—) is used instead. The desired reaction of the medium is adjusted by calculating the amount of acid or alkali required in terms of normal solution (N/1) for the total volume, and adding this amount directly to it. For example, if 2.3 c.c. of N/20 NaOH were required to neutralize a 5 c.c. sample of a freshly prepared broth, for 100 c.c. of this broth 2.3 c.c. of N/1 NaOH would be required (+ 2.3 per cent.). The reaction desired was + 1.0 per cent.; and as the difference between this and the reaction found (+ 2.3 per cent.) is equivalent to + 1.3 per cent., therefore, 1.3 c.c. of N/1 NaOH must be added to each 100 c.c. of the original broth for the correction.

After adding the required amount of alkali or acid to adjust the reaction, mix the medium thoroughly and place it in a steaming Arnold sterilizer for twenty to thirty minutes, if it is subsequently to be sterilized by the intermittent method in the Arnold sterilizer. If the subsequent sterilization is to be done in the autoclave, however, the preliminary heating also should be done in the autoclave at the same temperature and pressure, otherwise the final sterilization might cause a heavy precipitate to separate in the medium. The heating causes most of the precipitated phosphates to separate completely and they may be removed later by filtering. Correct the volume for evaporation, and again titrate samples. If the reaction is not within the required limits, make further corrections; mix; return to the steaming Arnold sterilizer for five to ten minutes, or boil for five minutes over flame; correct volume; then filter. The boiling or sterilizing of media usually causes an increase in acidity, especially in meat infusion broth, less so in meat extract broth; therefore, an amount of alkali slightly in excess of the estimated amount may be

added in adjusting the reaction. No definite rule can be given to cover this condition, since a number of factors enter which are subject to variation; the percentage excess must be determined for each particular method.

CLEARING OF MEDIA.

After the heating following the adjustment of reaction, most fluid media are sufficiently clarified by passage through double folded heavy filter paper. Gelatin and agar media are best filtered through layers of absorbent cotton. Make this filter by taking ordinary cheese cloth, wash thoroughly, fold to form two or three thicknesses about 12 x 12 inches in size; place on this a layer from roll of absorbent cotton about 10 x 10 inches; and fit the whole into an 8 inch funnel, packing down toward the outlet and to the sides. When this is in position, gently decant the hot medium into this filter without disturbing the arrangement. It may be necessary to pass the first portions of the medium through two or three times before the cotton filter becomes filled sufficiently to give a clear filtrate. In some cases this does not give the desired clearness, and it, therefore, becomes necessary to use another procedure. For this purpose, egg albumin is employed, usually the white of one or two eggs per liter. Whip up the egg white in a small amount of water and thoroughly mix with the medium, which has had its reaction adjusted and has been cooled to a temperature of 60 C. or less. Put the mixture into straight side enamel-ware vessels which may be placed either in the Arnold sterilizer or in the autoclave for the heating and coagulation of the albumin. The temperature should be brought up gradually to the point which will be used later in the sterilization of the medium, thus preventing further precipitation in the filtered medium. Use the autoclave for the coagulation of the egg white if the medium is to be sterilized by that method, and at the same pressure; otherwise use the Arnold sterilizer. Heat for thirty minutes, then remove from sterilizer; if the coagulum has formed at the surface, skim off; strain the hot medium through cheese cloth, folded to form four layers; press out the medium; correct volume; titrate; and adjust reaction if necessary. In such case heat at boiling point for five minutes before filtering. Finally, filter through a cotton filter prepared as outlined above.

In media prepared with meat infusion broth, instead of meat extract broth, the clarification may be carried out without the addition of egg white by extracting the meat in the first place at a temperature under 60 C.; then at a later stage bringing up to the boiling point and precipitating the coagulable proteins present. This method should be used when possible, and will be outlined later.

PREPARATION OF GLASSWARE FOR MEDIA.

It is essential that all test tubes, bottles, flasks, etc., which are to receive media should be thoroughly washed, freed from impurities, and well dried. Test tubes should be firmly stoppered with plugs of long fiber cotton (not absorbent), folded so that no stringy ends project into the tube. The plug should extend into the tube ¾ to 1 inch, depending on the size of the tube, and a free end should be left which will permit its ready removal. For large tubes, flasks, etc., it is well to cover the cotton plug with one or two layers of muslin or cheese cloth. All the glassware is sterilized in the dry heat oven for one hour at 170 C.

TUBING OF MEDIA.

Test tubes may be conveniently filled by connecting a discharging tube to the stem of a 6 inch funnel by means of a 3 inch soft rubber tube of suitable size which is closed by a stopcock. In filling the tubes care must be taken to prevent any of the medium from soiling the portion of the tube where the cotton plug might come in contact with it. The standard 6 x ⅝ inch test tube is used for most of the fluid media and for the liquefiable media which are to be slanted, 5 or 6 c.c. of the former or 8 to 10 c.c. of the latter being placed in each tube. The solid media, such as Loeffler's blood-serum medium, are put up in smaller tubes, which receive an amount sufficient to make a slant extending about one-half of the length of the tube; the same applies to the egg media.

Bottles (8 oz.) for blood culture work are filled about one-third full (75 c.c.).

STERILIZATION OF MEDIA.

In general, the intermittent method of sterilization causes less alteration of the constituents in media and is to be preferred when

time permits. All media containing sugars or other easily altered substances should be sterilized in this way. The Arnold sterilizer is used; it should be actively steaming when the media is placed in it, and time should be taken at that point.

Fluid media in test tubes, fermentation tubes, or small flasks should be sterilized for twenty minutes on each of three consecutive days; in the interval the media should be kept in a warm place. Liquefiable media, such as dextrose-agar in tubes, should be given a somewhat longer time period (not to exceed thirty minutes) on each of three consecutive days. When the media is in larger containers a proportionately longer time period should be allowed. When sterilization is carried out in the autoclave, thirty minutes at fifteen to eighteen pounds pressure are necessary if the medium is contained in test tubes or small vessels; one hour at the same pressure if larger containers are used.

SPECIAL DIRECTIONS

Immediately following this, methods for the preparation of the more commonly used culture media will be outlined. For those media not ordinarily used in routine work, the original sources of publication must be consulted.

Media may be grouped conveniently under three main divisions, based upon their physical character: liquid, liquefiable, and solid. This classification will be followed for convenience of reference.

I. LIQUID MEDIA

Litmus Milk. Separate the cream in fresh milk by placing the milk in a flask and heating it for fifteen minutes in a steaming Arnold sterilizer, and then putting it in the ice box for twelve to fifteen hours. After this, remove the lower portion from the surface cream layer by siphoning it into another flask. Fat-free milk can be procured from some milk dealers and is to be preferred when available. To the fat-free milk, add sufficient litmus solution to give a deep lavender color. Place about 10 c.c. in each tube, and sterilize in Arnold sterilizer for twenty minutes on each of three consecutive days. Milk should not be used if its reaction is more than + 1.5 per cent., unless neutralized with sodium hydroxide.

Dunham's Peptone Solution. Place 1000 c.c. of distilled or clear tap water in an enamelled stew-pan; add 10 gm. of peptone (American) and 5 gm. of sodium chloride; dissolve by boiling a few minutes; then filter through a double thickness of filter paper. The reaction should not exceed + 1.0 per cent. Sterilize either in the steaming Arnold sterilizer for twenty minutes on three consecutive days, or in the autoclave at fifteen pounds for thirty minutes.

Neutral Red Lactose Peptone. To a peptone solution prepared as outlined above, add 1 per cent. of lactose, and 1 per cent. of a saturated aqueous solution of neutral red. Dissolve sugar by heating; filter through filter paper, place in fermentation tubes, and sterilize for twenty minutes on each of three consecutive days, in Arnold sterilizer.

Nitrate Broth. To 1000 c.c. of water (ammonia free) add 1 gm. of peptone and 2 gm. of nitrite-free potassium nitrate. Heat until dissolved. Filter through double layer of filter paper; tube; and sterilize in Arnold sterilizer for twenty minutes on each of three consecutive days.

Plain Broth or Bouillon. Either meat extract or meat infusion may be used in the preparation of this medium. The use of the former simplifies the procedure and for ordinary purposes gives satisfactory results. Meat extract broth is prepared as follows: Place 1000 c.c. of water in enamel stew-pan; to this add 10 gm. of Liebig's meat extract, 10 gm. of peptone (American), and 5 gm. of sodium chloride, and dissolve by heat while stirring constantly. Measure volume, and add water for loss by evaporation. After mixing thoroughly, titrate, and adjust reaction to + 1.0 per cent. Place in a flask, and heat for twenty to thirty minutes in either the Arnold sterilizer or the autoclave, depending upon the method to be used for the final sterilization (see General Directions). After this, correct volume again, and take reaction; make corrections for reaction if necessary, and boil again for five minutes. Filter the solution through double layer of paper; tube; and sterilize in autoclave, or in Arnold sterilizer by the intermittent method. To prepare meat infusion broth select lean meat; remove all fat, tendons, and fascia; chop fine; add 500 gm. to 1000 c.c. of either distilled or clear tap water in suitable container. Place this in ice box and allow to macerate for eigh-

teen to twenty-four hours; or place it in a water bath and keep at a temperature of 55 to 60 C. for one hour, stirring occasionally. Following either of these procedures, strain through wet cheese cloth, express the juice from the meat, and bring the volume to 1000 c.c. Place this in an enamel vessel; bring temperature up to 50 C. over flame; add 10 gm. of peptone, and 5 gm. of sodium chloride; and stir until dissolved. Titrate samples and adjust the reaction to + 1.0 per cent. Proceed from this point as outlined for meat extract broth.

Sugar-free Broth. A meat infusion broth is prepared as outlined above up to the point immediately following the heating of the infusion in the Arnold sterilizer for twenty to thirty minutes. Then, instead of proceeding to readjust the reaction, filter the meat infusion through a layer of absorbent cotton and cheese cloth, press out, and bring to original volume. Cool the infusion to room temperature and inoculate each liter with a twenty-four hour broth culture of B. coli communis. Stopper the flask with cotton plug; place flask in incubator at 37 C. for about eighteen hours, then remove and put in autoclave for one hour at fifteen to eighteen pounds pressure. After the sterilization, restore volume; titrate samples; adjust reaction to neutral point; then heat in steaming Arnold sterilizer for twenty to thirty minutes. Filter through double thickness of filter paper; repeat until filtrate is clear. If the broth is not to be used at once as medium, place it in flasks, plug with cotton, and sterilize in the usual way by the intermittent method.

Carbohydrate Broth Fermentation Media. Sugar-free broth prepared as above is the base for the fermentation media. The substances most commonly used in this connection are the monohexoses: dextrose, galactose and levulose; the bi-hexoses: lactose, maltose, and saccharose; the polysaccharid: dextrin; and the polyhydric alcohol: mannite. These substances, which must be of the highest purity, are generally used in 1 per cent. concentration.

Place 500 c.c. of sugar-free neutral broth in clean flasks; add 5 gm. of the desired fermentable substance; and dissolve by heating gently if necessary. At once tube in sterile fermentation tubes, and immediately start sterilization in the Arnold sterilizer.

Heat for twenty minutes in flowing steam for three consecutive days.

Carbohydrate Serum-Water Fermentation Media. Ox blood serum is used in the preparation of these media. The blood is collected in covered sterile enamel-ware pails from the animal at the abattoir, care being exercised to exclude all unnecessary contamination. The freshly collected blood is allowed to clot before being moved.

Later, free the clot from the sides of the vessel with a sterile rod, place in ice box for twenty-four to thirty-six hours for separation of serum. Remove clear serum from clot by means of a large sterile pipette. If not clear, place in large centrifuge tubes, throw down cells, then remove the supernatant serum. Measure the serum and to this add two or three times its volume of distilled water—usually the proportion of one to three gives satisfactory results. Heat the mixture for fifteen minutes in the steaming Arnold sterilizer. Aqueous litmus solution may be added, if desired, until the fluid shows a deep transparent blue. Divide the finished serum into measured lots, and add to each the desired sugar in proportion of 1 gm. to 100 c.c. Dissolve, place in tubes, and sterilize in the Arnold sterilizer in the regular way.

Inulin Serum-Water. If the inulin is free from resistant spores, add it directly to the serum-water, prepared as outlined above, 1 gm. to each 100 c.c. But when resistant spores are present, sterilize the inulin first. Add 10 gm. of inulin to 750 c.c. of distilled water, place in autoclave and heat for fifteen minutes at fifteen pounds pressure. Remove and cool the sterile solution; then add 250 c.c. of clear ox blood serum and mix thoroughly. Add sufficient litmus solution to bring to a deep transparent blue color. Put about 10 c.c. in the ordinary culture tubes, and sterilize in the Arnold sterilizer for twenty minutes on three consecutive days.

Jackson's Lactose-Bile Medium. To 1000 c.c. of sterilized undiluted ox-gall (or 110 gm. of dry, fresh ox-gall dissolved in 1000 c.c. of water) add 10 gm. of peptone and 10 gm. of lactose. Dissolve by heating; filter through cotton until clear; divide in fermentation tubes and sterilize in the Arnold sterilizer in the usual way. In case large quantities of material, such as milk or

water, are used for inoculation, place 40 c.c. of the medium in large fermentation tubes and sterilize as above.

Dextrose Broth. This medium, when used only for cultural purposes, need not be made from fermented infusion broth. Either meat extract or meat infusion broth prepared in the usual way may be used. The reaction should be adjusted so that it is neutral to phenolphthalein, although for some purposes it may be + 0.2 to + 0.5 per cent. To 1000 c.c. adjusted broth add 10 gm. of dextrose; dissolve; heat twenty minutes in the steaming Arnold sterilizer; and filter through double layers of filter paper. Tube and sterilize in the Arnold sterilizer for twenty minutes on three consecutive days.

Calcium Carbonate Dextrose Broth. Prepare dextrose broth as above, using meat infusion rather than meat extract broth as the base. To this add 1 per cent. powdered calcium carbonate (previously sterilized in hot air oven) or place small pieces of marble in the tubes, and add the dextrose broth. Sterilize as with the dextrose broth.

Dextrose Ascitic Broth. Prepare dextrose broth as above; tube; and sterilize in the usual way. To each tube add, by means of a sterile pipette under aseptic precautions, clear sterile ascitic fluid in the proportion of one part of the ascitic fluid to two or three parts of the dextrose broth. Place the tubes in the incubator at 37 C. for twenty-four to thirty-six hours to test their sterility.

II. LIQUEFIABLE MEDIA

Nutrient Gelatin. Meat infusion, or meat extract broth may be used in the preparation of this medium. Take 1000 c.c. of meat infusion broth, add 10 gm. of peptone, 5 gm. of sodium chloride, and 120 gm. of best Gold Label gelatin, and dissolve the ingredients at a temperature under 60 C. Titrate samples; adjust reaction to a neutral point (phenolphthalein); place in flask; and heat in steaming Arnold sterilizer for thirty minutes. After heating, correct volume; titrate samples; and adjust the reaction to + 1.0 per cent. If additional alkali has been required, heat again at boiling point for five minutes, then filter through absorbent cotton, repeat until clear. Titrate the medium and note the reaction.

If meat extract is used, add to 1000 c.c. of water, 10 gm. of Liebig's extract, 10 gm. of peptone, 5 gm. of sodium chloride, and 120 gm. of Gold Label gelatin, and dissolve under 60 C. Titrate samples; adjust reaction to neutral point; then add the whites of one or two eggs beaten up in a small amount of water; shake up in flask; and heat in steaming Arnold sterilizer for thirty minutes. Correct volume; titrate samples; adjust reaction to + 1.0 per cent. and heat at boiling point for five minutes if additional alkali has been required. Filter the medium through absorbent cotton, repeating the process until clear. Nutrient gelatin should never be heated more than is absolutely necessary. Sterilize in Arnold sterilizer for thirty minutes on each of three consecutive days.

Plain Nutrient Agar. Meat infusion or extract of meat broth may be used in the preparation of this medium. When the meat infusion broth is desired, it is prepared by making the infusion double strength (500 gm. of meat to 500 c.c. of water). In such case add 10 gm. of peptone and 5 gm. of sodium chloride to 500 c.c. of this infusion, and dissolve at a temperature under 60 C. To 500 c.c. of water add 20 gm. of thread agar and dissolve, either by boiling over a free flame, or, better, by placing in autoclave and heating for thirty minutes under fifteen pounds pressure. Cool the agar solution to 60 C. or slightly under; combine with the infusion broth at same temperature; bring total volume to 1000 c.c.; mix thoroughly; titrate samples; and adjust reaction to neutral point. Place this in the autoclave, and heat for thirty minutes at fifteen pounds pressure. Titrate samples; adjust reaction to about + 0.7 per cent.; and boil five to ten minutes, after which filter the medium through absorbent cotton, repeating until clear. Sterilize the medium either in the autoclave for thirty minutes at fifteen pounds pressure, or in the Arnold sterilizer for twenty to thirty minutes for tubes (longer for larger containers) on three consecutive days. Meat infusion media become acid on heating, so that a titration should be done after final sterilization; the reaction should be near + 1.0 per cent. A meat extract base is not so prone to become acid on heating, therefore, when this is used, adjust to desired reaction directly.

The preparation of plain nutrient agar with meat extract instead of the meat infusion is much more easily carried out, and

answers very well in most cases. To 1000 c.c. of water add 10 gm. of Liebig's meat extract, 10 gm. of peptone, 5 gm. of sodium chloride, and 20 gm. of thread agar. Place this mixture in autoclave and heat for thirty minutes at fifteen pounds pressure to dissolve. Bring to original volume; titrate samples; adjust reaction to + 0.9 per cent.; cool to 60 C.; then add the whites of one or two eggs dissolved in a small amount of water; and mix thoroughly. Again heat in the autoclave as before, after which filter the hot agar medium through absorbent cotton until clear. Tube; and sterilize either in the autoclave or Arnold sterilizer in the usual way. The final reaction of the sterilized medium should be about + 1.0 per cent.

In some cases a "salt-free agar" is desired. This is prepared as above with the exception that the sodium chloride is omitted.

Dextrose Agar. Prepare plain nutrient agar as outlined above; adjust reactions from neutral to + 0.5 per cent., depending upon the particular use desired. To the clarified, filtered agar add 10 gm. of dextrose to each 1000 c.c. Tube in amounts for making slants and for plating; sterilize for twenty minutes on each of three consecutive days, in Arnold sterilizer.

Blood Agar. This medium is prepared by adding sterile, defibrinated blood to plain nutrient agar, either by mixing at a low temperature or by spreading over the surface of agar slants. The reaction of the agar for this purpose should be near the neutral point. Dissolve by heat the prepared agar medium and, by means of a pipette, measure 4.75 c.c. into each test tube. Sterilize in autoclave in the usual way. Cool the tubes to 50 C. in water bath, and to each add 0.25 c.c. of defibrinated blood by means of sterile pipette under aseptic conditions. Mix thoroughly, then slant to harden. After they are well hardened, place tubes in incubator (37 C.) for twenty-four to thirty-six hours to test sterility. If surface tubes are to be prepared, use plain agar slant tubes to which add one to two drops of defibrinated blood, spread well over the surface, and incubate to test sterility.

Rabbits' blood gives very satisfactory results in blood agar medium. To collect it bind a healthy animal on a suitable holder, and anesthetize it thoroughly with ether. Shave the neck over throat region, clean carefully, and sterilize skin as far as possible with 1:1000 solution of bichloride of mercury, or by painting with tincture of iodine. By means of sterile

instruments, make a longitudinal incision about 1.5 to 2 inches through the skin, and pull this aside to expose the muscles and trachea; divide the muscles by blunt dissection; and expose the carotid artery. Ligate the distal end of the artery, then clamp the proximal end and pass a ligature under to tie in position a glass cannula inserted in the artery through an opening made by means of fine tipped scissors midway between the two points indicated. When the sterile glass cannula is inserted and tied in the artery, remove the clamp, thus allowing the blood to flow out into sterile tubes or flasks containing coils of copper wire. Defibrinate the blood by shaking in these containers from ten to fifteen minutes. With care, the blood can be collected aseptically without difficulty, and can then be kept in a sterile condition in the ice box for future use.

Dextrose Ascitic Agar. Prepare dextrose agar as outlined above, and after final sterilization in the Arnold sterilizer cool in water bath to 60 C. By means of a sterile pipette, add sterile ascitic fluid in the proportion of one part of ascitic fluid to three parts of dextrose agar. Incubate at 37 C. for twenty-four to thirty-six hours to test sterility.

Starch Agar. Prepare beef infusion in the usual way, without the addition of peptone or salt. To 1000 c.c. of the infusion add 15 to 17.5 gm. of agar. Dissolve the agar by heating, correct volume, clarify, and filter. Titrate and adjust so that the final reaction will be 0.2 to 0.5 per cent. acid to phenolphthalein. Grind up 10 gm. of corn starch in a mortar with a little of the agar to avoid lumps; then add this to the main portion and boil for a few minutes. Tube in amounts for plating purposes, also for slants. Sterilize in autoclave for thirty minutes at a pressure not exceeding fifteen pounds.

Sodium Glycocholate Agar. To 1000 c.c. of water add 15 gm. of peptone and 15 gm. of agar, and dissolve by heating, either by boiling over flame, or in autoclave for thirty minutes at fifteen pounds pressure. Cool to 60 C., add 35 gm. of lactose and 5 gm. of sodium glycocholate; titrate; and adjust reaction to + 1.0 per cent. Then add the white of one or two eggs, (whipped up in a small amount of water); mix thoroughly; and place in steaming Arnold sterilizer for thirty minutes. Titrate again, after restoring to original volume, and correct reaction if necessary. In case more alkali must be added to readjust the reaction, heat again for five to ten minutes in the Arnold sterilizer, then filter through a layer of absorbent cotton, repeating until clear. Tube,

and sterilize, for twenty to thirty minutes on each of three consecutive days, in Arnold sterilizer.

Glycerin Agar. To 1000 c.c. of plain nutrient agar, prepared in the usual way, and adjusted to neutral reaction, add 50 c.c. of chemically pure glycerin. Tube, and sterilize in Arnold sterilizer for twenty to thirty minutes on each of three consecutive days.

Endo's Fuchsin Agar. To 1000 c.c. of water add 30 gm. of agar, 10 gm. of peptone, 10 gm. of Liebig's extract of beef, and 5 gm. of sodium chloride; dissolve by boiling or heating in the autoclave for thirty minutes at fifteen pounds pressure. Cool to 60 C.; add egg white to clear in the usual way, and filter to clearness through absorbent cotton. Correct volume, and make neutral to litmus (fresh papers) by the addition of sodium hydroxide; then add 10 c.c. of a 10 per cent. solution of sodium carbonate. Add 10 gm. of lactose, and 5 c.c. of saturated alcoholic solution of fuchsin, freshly filtered; mix thoroughly; add 10 per cent. sodium sulphite solution until the color just disappears on solidifying; usually about 25 c.c. is required Add about 15 c.c. of the medium to each tube, and sterilize in the Arnold sterilizer for twenty to thirty minutes by the regular intermittent method. Store in a dark place.

Kendall's modification of this method may be carried out as follows: Prepare 1000 c.c. of meat extract in usual way; make reaction slightly alkaline to litmus; divide in bottles (100 c.c. to each); sterilize, and store for stock. Mix medium and prepare fresh plates each time before using as follows: Add to each bottle 1 gm. of lactose; heat until dissolved, thus sterilizing the lactose; then add 1 c.c. of decolorized fuchsin, which has been prepared by adding 1 c.c. of saturated alcoholic solution of fuchsin to 10 c.c. of a 10 per cent. aqueous solution of sodium sulphite and heating in the Arnold sterilizer for twenty minutes. Pour the finished medium into plates and allow to harden. Place plates in the incubator to dry for thirty minutes, arranging them so as to protect them from dust.

North's Agar Gelatin Medium. Extract 500 gm. of lean chopped beef or veal with 500 c.c. of water by maceration for eighteen hours. Strain and press out juice; then add 10 gm. of agar, 20 gm. of Gold Label gelatin, 20 gm. of peptone, 5 gm. of

sodium chloride, and sufficient water to bring volume to 1000 c.c. Dissolve by heat and adjust the reaction to the neutral point for phenolphthalein. Heat again in the Arnold sterilizer, then filter through absorbent cotton until clear. Tube and sterilize in the Arnold sterilizer, by the intermittent method. If desired, 1 per cent. dextrose, which is more favorable for certain organisms, may be added.

III. SOLID MEDIA.

Plain Potato Medium. Select large potatoes and scrub them thoroughly with small brush under running water. By means of an apple corer, cut cylinders from the cleaned potato. Remove skin from each end of cylinder, and by an oblique cut divide into wedge-shaped portions with good butts. Drop them in clear running water, and wash for several hours. After this, drop a wedge, large end downward, into special potato tubes, or the ordinary straight tube, with a small wad of moist absorbent cotton in the bottom, sterilize in the Arnold sterilizer in flowing steam for twenty minutes on three consecutive days.

Glycerin Potato Medium. Prepare potato wedges as mentioned above, and place them in a 10 per cent. aqueous glycerin solution for several hours. Drop the wedges and a few drops of the glycerin solution into potato tubes and sterilize by placing in steaming Arnold sterilizer for twenty minutes on three consecutive days.

Loeffler's Blood-serum Medium. Separate clear serum from either clotted sheep or ox blood as outlined under the preparation of serum-water media. Prepare 1 per cent. dextrose broth from meat (beef or veal) infusion, and adjust reaction to neutral point with phenolphthalein as indicator. Mix three parts of the serum with one part of dextrose broth and place in small sized culture tubes a sufficient amount for slanting. Put these in a slanting position in a Koch serum coagulator and very gradually heat until a temperature of 90 C. is reached; hold the temperature at this point until the medium is completely solidified. In the absence of a Koch serum coagulator, the medium may be solidified either in a dry heat sterilizer, an autoclave, especially the horizontal type with steam jacket, or even an Arnold sterilizer. Success depends upon raising the temperature very slowly, and not

exceeding 90 to 95 C., thus preventing the formation of bubbles in the medium. After solidification of the medium, sterilize on the second and third days by placing in the Arnold sterilizer; slowly heat until the boiling point is reached, and hold there for twenty minutes.

Lubenau's Glycerin Egg Medium. Select ten perfectly fresh eggs, clean thoroughly with water, then wash with 5 per cent. carbolic acid. Allow to dry partially in the air; then dry both ends in the flame of a Bunsen burner. By means of a sterile (flamed) sharp pointed forceps make an opening of about 0.5 inch diameter through shell and membrane at one end; at the other end make a smaller opening through shell, if possible without breaking the membrane. Place lips to small opening and gently blow out contents of the egg into a sterile wide-mouthed flask or bottle. To the total contents of the ten eggs add 200 c.c. of neutral 5 per cent. glycerin broth. Mix thoroughly by gently shaking or stirring with sterile glass rod. Divide into test tubes by sterile plugged pipette; slant in serum coagulator; and heat at 70 C. for fully two hours on each of two consecutive days. If the medium is too dry after the sterilization, add two or three drops of sterile water to supply moisture. Incubate at 37 C. for twenty-four to thirty-six hours to test sterility.

PREPARATION OF STAINING FLUIDS, STAINING TECHNIQUE, ETC.

In general, the anilin dyes commonly used in the staining of bacteria are made up in concentrated or saturated solutions, either in distilled water or in 95 per cent. alcohol. Dilutions are made from such "stock solutions" after filtration for use in routine staining methods. The preparation of staining fluids may be conveniently considered under the following headings: (1) Concentrated Stock Solutions; (2) Simple Staining Solutions, Mordants, Etc.; (3) Special Stains and Staining Methods.

I. CONCENTRATED STOCK SOLUTIONS

Since considerable variation, both chemical and physical, exists between different samples of dyes appearing under the same name, no constant minimum can be given for the prepara-

tion of a saturated solution. The amounts given for the preparation of the following concentrated solutions are in part based upon the table prepared by Wood.

If a completely saturated solution is desired, the dye must be added until no more is soluble, as indicated by a slight excess of residue. The dyes used should be of the highest purity, especially prepared for bacteriological or medicinal purposes. Usually the dye is placed in the solvent at room temperature and dissolved by shaking thoroughly a number of times during several days. When desired for the preparation of the staining fluid, portions of the solution are removed and passed through soft filter paper.

Bismarck brown (Vesuvin), conc. aqueous solution. Add 0.5 gm. of Bismarck brown to each 100 c.c. of boiling distilled water. *Note:* A small piece of camphor may be added to preserve the solution.

Fuchsin (basic), conc. alcoholic solution. Add 3.0 gm. to each 100 c.c. of 95 per cent. alcohol, and dissolve.

Gentian violet, conc. alcoholic solution. Add 4.8 gm. to each 100 c.c. of 95 per cent. alcohol, and dissolve.

Methylene blue, conc. alcoholic solution. Add 7.0 gm. to each 100 c.c. of 95 per cent. alcohol, and dissolve.

II. SIMPLE STAINING SOLUTIONS, MORDANTS, ETC.

Acid alcohol. Add 2 gm. (volume 1.7 c.c.) of hydrochloric acid (sp. gr. 1.19) to 80 or 90 c.c. of 80 per cent. alcohol; mix; then bring volume up to 100 c.c. with the diluted alcohol. *Use:* Decolorizing agent. See stain for "acid-fast" bacilli.

Anilin-water. Add 5 c.c. of anilin oil to 125 c.c. of distilled water, thoroughly shake in flask, then filter through a double layer of filter paper well moistened with distilled water to retain undissolved anilin. *Use:* Preparation of anilin-water gentian violet. See Gram's stain.

Bismarck brown stain. Add 10 c.c. of filtered stock solution of the dye to 90 c.c. of distilled water. *Use:* As a counterstain apply one-half to one minute in Neisser or Gram methods.

Carbolic acid solution (5 per cent.). Add 5 gm. of chemically pure carbolic acid (phenol) to 95 c.c. of distilled water. Shake until dissolved. Filter. *Use:* Preparation of carbolfuchsin.

Copper sulphate solution. Dissolve 20 gm. of chemically pure

copper sulphate by shaking with about 75 c.c. of distilled water, then bring volume to 100 c.c. with water. *Use:* See Hiss capsule stain.

Fuchsin (basic), aqueous. Add 5 c.c. of filtered alcoholic stock solution of basic fuchsin to 95 c.c. of distilled water. *Use:* As a counterstain apply one-half to one minute.

Fuchsin, carbol. Add 10 c.c. of filtered alcoholic stock solution of basic fuchsin to 90 c.c. of 5 per cent. aqueous solution of chemically pure carbolic acid. *Use:* As a simple stain one-half to one minute. Also see spore stain and acid-fast bacilli stain.

Gentian violet, aqueous. Add 5 c.c. of filtered alcoholic stock solution of gentian violet to 95 c.c. of distilled water. *Use:* See Hiss capsule stain.

Gentian violet, anilin-water. Add 14 c.c. of saturated alcoholic solution of gentian violet to 126 c.c. freshly prepared anilin-water. Filter. *Note:* This stain should be freshly prepared every three to four days. *Use:* Principally in Gram's staining method.

Gram's iodine solution. Place 1 gm. of iodine and 2 gm. of potassium iodide in glass or porcelain mortar, and rub to a fine powder, gradually add a small amount of distilled water until all is dissolved, then bring total volume to 300 c.c. with distilled water. Filter. *Use:* Principally in Gram's staining method.

Potassium hydrate solution (1:10,000). Dissolve 1 gm. of potassium hydroxide in 100 c.c. of distilled water. Take 1 c.c. of the 1 per cent. solution just prepared and add 99 c.c. of distilled water to give the required dilution. *Use:* Preparation of Loeffler's methylene blue stain.

Sulphuric acid solution, (5 per cent.) Add slowly 5 gm. (volume, 2.7 c.c.) of sulphuric acid (sp. gr. 1.84) to 80 to 90 c.c. of distilled water, then as the solution cools bring total volume to 100 c.c. *Use:* Decolorizing agent. See Ziehl-Neelsen staining method.

III. SPECIAL STAINS AND STAINING METHODS

Capsule stain (Hiss). Smear material, such as sputum, chest fluid, etc., on clean cover slip or slide; if it contains no albuminous substance, place a drop of beef-blood serum on the slide, and spread. Dry in air and fix with heat. Cover with aqueous

gentian violet and heat over full flame until steam rises for a second. Wash off staining fluid with copper sulphate solution. Blot dry without washing in water, and examine.

Flagella stain (Loeffler). Use young growth from agar culture by gently mixing a small amount in a large drop of physiological salt solution on a perfectly clean, flamed cover slip. Dry quickly over flame. Fix in flame, but do not overheat. Prepare a mordant solution as follows:

Aqueous solution (20 per cent.) tannic acid,	10 c.c.
Saturated solution of ferrous sulphate,	5 c.c.
Saturated alcoholic solution of fuchsin,	1 c.c.

Filter at once after mixing, and use while fresh.

Pour mordant solution over fixed material and gently warm for one to five minutes. Wash in water. Stain with freshly filtered anilin-water gentian violet for one to two minutes; wash in water; dry; mount in balsam.

Gram's staining method. Make preparations of material or culture on cover slip (or slide); dry in air; fix in flame; and allow to cool. Cover with anilin-water gentian violet stain for five minutes at room temperature. Wash off staining fluid with Gram's iodine solution, allowing it to act for one minute. Wash off iodine solution with 95 per cent. alcohol, continuing this decolorizing process until the alcohol shows only a very faint violet tint. Wash in distilled water; counter-stain with either aqueous fuchsin or Bismarck brown solution for one minute; blot until dry.

Loeffler's alkaline methylene blue stain. Add 30 c.c. of saturated alcoholic solution of methylene blue to 100 c.c. of 1:10,000 aqueous solution of potassium hydrate. *Note:* Much better results are given if this stain is allowed to stand some weeks before using. *Use:* Apply stain for one minute or more to fixed preparation.

Neisser's acid methylene blue stain. Add 1 gm. of methylene blue to 20 c.c. of absolute alcohol, 50 c.c. of glacial acetic acid, and 1000 c.c. of distilled water; dissolve; and filter. *Use:* Apply to fixed preparation for two to five seconds, wash in water, and counterstain with Bismarck brown solution for five to ten seconds. Polar bodies appear blue, bacillary bodies brown.

Pappenheim's stain. In 100 c.c. of filtered saturated solution of methylene blue in absolute alcohol, dissolve 1 gm. of corallin (rosolic acid); then add 20 c.c. of glycerin. *Use:* This method is especially useful in the examination of materials for tubercle bacilli from sources where the smegma or other acid-fast bacilli may be present. Make smears of the material on slide and fix by heat in the usual way. Add carbolfuchsin solution and heat on hot plate or over flame until steam rises for two to three minutes. Pour off stain; then place in staining dish containing Pappenheim's stain; and allow to stand for one-half hour, occasionally shaking the dish. Remove, wash in water, and dry.

Spore stain. Make smears of old culture on cover slip or slide; dry, and fix with heat. Add carbolfuchsin; heat until staining fluid gently steams for three to five minutes; then wash in acid alcohol for about one minute. Wash in water; then counterstain with Loeffler's methylene blue for one-half minute; wash; dry. Spores appear red, while the body of the organism is blue.

Huntoon employs another method which is simpler, giving the double stain in a single step. Make a staining mixture as follows: Dissolve 4 gm. of acid fuchsin in 100 c.c. of 2 per cent. acetic acid; likewise dissolve 2 gm. of methylene blue (Grübler's) in 100 c.c. of 2 per cent. acetic acid. Mix the two solutions, filter to remove the resulting precipitate, and reserve the filtrate for staining. Cover the prepared, fixed smear with the staining solution, and steam over flame for one to two minutes. Wash in running water, dry and examine. Spores appear red, while the bodies of the bacteria stain blue.

Ziehl-Neelsen stain. Ziehl's carbolfuchsin is made as follows: In 100 c.c. of distilled water dissolve 1 gm. of basic fuchsin and 5 gm. of carbolic acid; filter the solution, then to the filtrate add 10 c.c. of absolute alcohol. Make thin smears of material for examination on cover slip or slide, dry and fix with heat; cover with the staining solution and heat until it gently steams for five minutes. Pour off stain, and decolorize by covering the stained preparation with 5 per cent. solution of sulphuric acid; immediately pour it off; repeat the procedure again; and wash in water, then in 90 per cent. alcohol, until no more color is dissolved. Others prefer to wash with acid alcohol until the preparation is practically decolorized. In either case, wash with water, then

counterstain with Loeffler's methylene blue solution for about one-half minute. Wash with water, dry and mount.

PREPARATION OF DISINFECTING SOLUTIONS

Carbolic acid, stock solution. Dissolve crystals of pure phenol by placing container, after the removal of stopper, in water-bath, and applying heat. To each 95 c.c. of the liquified phenol add 5 c.c. of glycerin, mix thoroughly, and place in glass-stoppered bottle. *Use:* Dilute for disinfecting solution.

Carbolic acid (C. P.) 5 per cent. solution. To 52.5 c.c. of stock solution of carbolic acid add sufficient water to bring total volume to 1000 c.c., and mix. Or weigh out 50 gm. of phenol crystals; add sufficient water to bring to 1000 c.c.; and dissolve.

Carbolic acid (crude) 3 per cent. solution. Dissolve about 25 gm. of commercial green soap in 400 to 500 c.c. of hot water, cool, then add 30 c.c. of crude carbolic acid; thoroughly mix; then add water to bring total volume to 1000 c.c.; mix.

Cresol, 2.5 per cent. solution. Add 25 c.c. of compound solution of cresol (U. S. P.) to 975 c.c. of hot water to dissolve.

Iodine, tincture (U. S. P.). Weigh out 70 gm. of iodine, 50 gm. of potassium iodide, place in mortar, rub up quickly into powder form, then place in glass-stoppered bottle. To this add in small amounts 95 per cent. alcohol, and shake until powder is dissolved. Finally add sufficient alcohol to bring the total volume to 1000 c.c.

Mercuric chloride, stock solution. Place 40 gm. of bichloride of mercury in a glass-stoppered bottle, add 1000 c.c. of distilled water, and shake until the salt is completely dissolved. *Use:* Dilute for disinfecting solution.

Mercuric chloride, 1:1,000 solution. To each 25 c.c. of stock solution of mercuric chloride add sufficient water to bring to 1000 c.c. Add concentrated methylene blue solution until the solution shows a light blue tint.

PREPARATION OF MISCELLANEOUS SOLUTIONS

Physiological salt solution, No. 1. Dissolve 8.5 gm. of chemically pure sodium chloride in 1000 c.c. of *freshly* distilled water, and filter through a double layer of filter paper. Place 125 c.c.

in each of eight 250 c.c. flasks, tightly stopper with cotton plugs covered with two layers of cheese cloth, cap with one or two layers of white paper. *Note:* Flasks must be thoroughly cleaned and then rinsed with two or three washings of freshly distilled water before the salt solution is placed in them. Sterilize the flasks with the contained salt solution in the autoclave at 15 lbs. pressure for one hour. When cool, label and store for use. *Use:* Diluent for therapeutic and diagnostic materials used in wards, for transfusion tests, etc.

Physiological salt solution, No. 2. Dissolve 9 gm. of chemically pure sodium chloride in 1000 c.c. of *freshly* distilled water in clean flask. Prepare just before using. *Use:* Washing of sheep corpuscles, preparation of corpuscle suspension, and in the dilution of active substance in connection with the Wassermann reaction. It need not be sterilized for this purpose.

Sodium citrate solution, No. 1. Dissolve 1 gm. of chemically pure sodium citrate and 0.85 gm. of chemically pure sodium chloride in 100 c.c. of distilled water. Filter; place in tubes; plug tightly with cotton; then sterilize in steaming Arnold sterilizer for twenty minutes on each of three consecutive days. *Use:* In collection of small amounts of blood for blood cells.

Sodium citrate solution, No. 2. Dissolve 10 gm. of chemically pure sodium citrate and 0.85 gm. of chemically pure sodium chloride in 100 c.c. of distilled water. Filter; tube; and sterilize as above. *Use:* To prevent coagulation of freshly drawn blood add one part of citrate solution to nine parts of whole blood. Mix well.

BACTERIOLOGICAL TECHNIQUE.

GENERAL DIRECTIONS

IMPORTANT: ALWAYS BURN OFF PLATINUM NEEDLE IN FLAME OF BUNSEN BURNER JUST BEFORE AND AFTER USING.

WATCH TEMPERATURE OF INCUBATOR CAREFULLY; KEEP IT AT 37 C. IF POSSIBLE, NEVER ALLOW IT TO APPROACH 40 C.; TOO LOW A TEMPERATURE IS LESS SERIOUS.

Cultures, materials for culture, and smears are generally brought to the Bacteriological Laboratory, but in some cases, when so desired, they may be taken by the bacteriologist in the ward.

The patient's name, the ward, the nature of specimen, and a note as to any special examination ordered should be entered in the bacteriological day book as soon as the specimen is received, for example: "Norrie III. Smith, John. Sputum for *B. influenzae.*" It is well also to record the hospital case number to prevent confusion, for instance, when two patients with the same name are in the same ward, as has happened. Later, the diagnosis and findings are written in the book fully and clearly. The result of the examination is entered by the bacteriologist in the ward pathological record book under date of entry, with date on which culture was made, in parenthesis, giving the patient's name. A provisional report is made as soon as possible; thus, under January 2 "Smith, John. Culture from sputum (January 1) shows small Gram-negative bacilli." Under January 6, a final report is made: "Smith, John. Culture from sputum (January 1) shows *B. influenzae.*"

Exudates, etc., should be inoculated on culture media as soon as received and at once placed in the incubator. Cultures in culture media also should be placed in incubator immediately on reaching the laboratory. Exudates not taken sterilly, when there is danger that the characteristic organism may be overgrown (for example, pus from supposed tuberculous sinus for inoculation into a guinea-pig), are placed in the icebox until the bacteriologist is ready to inoculate.

CARE OF LUER SYRINGE.

If the syringe has been used for infectious materials, disinfect, either by boiling or by immersing in 2.5 per cent. cresol solution, depending upon the nature of material. After disinfecting wash in water until clean. If water fails to remove blood, etc., use the sulphuric acid-potassium bichromate mixture, then wash thoroughly in running water. Dry; place plunger and barrel separately in large test tubes containing a wad of gauze in the bottom; plug tubes with cotton covered with two layers of gauze; place in dry heat oven; and sterilize at once. To prevent mistakes, never plug the tubes until just before placing them in the sterilizer. Sterilize for 1 hour at 170 to 180 C.

CARE OF HYPODERMIC NEEDLES.

After using with infectious materials (exudates, blood, etc.) wash needle while on syringe with 2.5 per cent. cresol solution by drawing in and discharging the solution several times; then boil in water. After using for injection of culture, disinfect needle at once by boiling. When disinfected, clean out the hub with a bit of cotton on forceps, and the lumen with fine wire. Sharpen points on soft stone, and polish with jeweler's emery cloth, or "Crocus" cloth. Dry lumen by passing first alcohol, then ether, through the needle. Place point downward in small test-tube containing a plug of gauze in the bottom. Do not plug the opening of the tube until just before sterilizing. Sterilize with dry heat at 170 to 180 C. for one hour.

ANIMAL INOCULATIONS.

Animal inoculations are made only when ordered. The procedure depends upon the nature of the material which is supplied. Solid materials and coagula from serous fluid generally are imbedded beneath the skin; sometimes they can be emulsified by rubbing them with sterile salt solution in a sterile tube, or they can be dissolved in 2 per cent. antiformin, and then injected. All fluids are injected into the animal by means of a sterile all-glass syringe with suitable needle. If there is a large quantity of fluid (urine, chest fluid, etc.) centrifuge it, remove the supernatant portion (disinfect), then take up the sediment in a syringe and inoculate the animal. Guinea-pigs are used for the recognition of tubercle bacilli; the inoculations are generally made

subcutaneously in the inguinal region. Mice are used to identify pneumococci by subcutaneous inoculation at root of tail.

Embedding Solid Materials Subcutaneously. Sterilize by boiling 3 or 4 straight forceps; 2 or 3 pairs of small scissors, needle forceps, curved surgical needles, etc. Place animal under ether anesthesia; shave the lower portion of the abdomen; then paint the dry skin with tincture of iodine. Grasp skin with sterile forceps forming a longitudinal fold over prepared area, with small sterile scissors cut through this fold to a depth of 0.75 to 1 cm., thus giving a transverse incision through the skin of 1.5 to 2 cm. in length. Thrust closed scissors (3 to 4 cm. blade) through opening downward between skin and muscular wall toward inguinal region about 5 cm. distance, then open blades, thus making a small subcutaneous pocket, close, and remove. With sterile forceps grasp edge of the skin at lower margin of the incision, raise from body wall, and by means of sterile forceps take up solid mass for inoculation and thrust under skin to bottom of pocket, release and remove forceps. Sew opening by approximating edges with forceps, and running close continuous stitch with cat-gut; handle needle with forceps to prevent infection. Clean the area with 5 per cent. carbolic acid.

Small openings may be closed by approximating edges of incision, drying the skin, painting over with collodion, covering with a very thin layer of absorbent cotton and over this placing another layer of collodion.

Sterilize all instruments by boiling immediately after using.

Inoculation by Injection. In general, fluids are injected subcutaneously into animals, but in some instances may be inoculated directly into the peritoneal cavity or into the blood stream. The hypodermic syringe used for inoculation should be sterilized by boiling for 15 to 20 minutes. For subcutaneous inoculation, the animal is prepared as outlined above. Take up the material in syringe through the needle; inject by puncturing the skin and passing the point of the needle toward the inguinal region, and discharge the contents between skin and body wall about 3 to 4 cm. from the point of entrance. Withdraw needle; disinfect puncture point with tincture of iodine; seal with collodion if necessary to prevent leakage.

In making an intraperitoneal injection, prepare the animal in the usual way; pinch up abdominal wall; and pass needle

obliquely through both skin and wall to avoid puncturing abdominal viscera. After injection, disinfect needle puncture. Injection of fluids directly into the circulation of animals is usually done by puncture of heart or into superficial veins. In the case of guinea-pigs, the heart method is most commonly used. Place the animal under ether anesthesia; remove the hair from chest by shaving; and disinfect skin with tincture of iodine. Pass the needle of syringe containing the material through chest wall just to right of sternum about third intercostal space, direct downward and backward about 3 to 4 cm. If the needle enters the heart, blood may be drawn into the syringe. Slowly eject contents from syringe. In larger animals, such as rabbits, shave hair from upper surface at outer margin of the ear; and disinfect with 5 per cent. carbolic acid. The marginal vein will be visible. Inject material in the usual way after passing needle of syringe through skin and into vessel, pointing toward the base of the ear. After removal of needle from vessel, place small wad of cotton over puncture point at once, holding the cotton in place with small artery forceps to prevent bleeding; remove later and sterilize by painting area with tincture of iodine.

Examination of Inoculated Animals. The attendant in charge of the animal room daily observes all the inoculated animals, and reports the results to the bacteriologist. All such animals which die are placed in a tray, and sent to the laboratory for examination. In general, to perform an autopsy on a small animal, such as a guinea-pig or mouse, proceed as follows: Sterilize by boiling 3 or 4 forceps, and the same number of scissors and scalpels. Also have an extra instrument sterilizer at hand to receive the infected instrument after using. Place the animal back downward on a board (about 15 x 25 cm.), distend legs and nail down by long tack through each foot. Do this in a large tray to prevent possible spreading of infectious material, and disinfect hands after handling the animal. Wash the skin of the animal with 5 per cent. carbolic acid, or 2.5 per cent. cresol solution. Lift skin in median line in pelvic region with forceps. With sterile scissors cut through skin only, and extend incision upward to neck. From each extremity of the median incision cut outward to each leg. Dissect skin from body with sterile scalpel, retracting with forceps. Examine site of injection (if subcutaneous), regional lymph-nodes, etc., and make cultures and

smears from subcutaneous exudate if present. Place instruments in reserve sterilizer. Make incision through abdominal wall in median line from pelvic region upward to diaphragm with sterile scissors. Make any necessary cultures or smears, and examine organs, etc. Open thorax with sterile heavy scissors by cutting through ribs from lower margin upwards on both sides laterally to the sternum; free the diaphragm; remove the wedge-shaped plate; then make cultures and smears (if necessary) and examine organs. To make cultures and smears from heart blood, place a sterile wire behind heart; tip forward; cut tip of ventricle with sterile scissors; then take blood with sterile Pasteur pipette or platinum loop; and proceed in usual way.

The routine procedure in a post-mortem examination depends much upon the particular type of organism under consideration. In the case of a guinea-pig inoculated with material suspected of containing the tubercle bacillus, the animal if it survives is usually killed five to six weeks after injection and examined. At autopsy examine for tubercles, especially at the site of injection and in the regional lymph-nodes, spleen, liver, lungs, and peritoneum. Make smears from tubercles found, stain, and examine for the bacillus. Take tissue from organs showing suspicious lesions, prepare stained sections and examine microscopically. Examine guinea-pigs dead from inoculation of material showing diphtheria-like organisms, particularly as to the character of the exudate at the site of injection, making smears and culture; examine also the peritoneal fluid; and the condition of the adrenals, noting whether they are hemorrhagic or not.

Animals inoculated with materials which are to be tested for other types of organisms are handled in similar manner at autopsy. Smears and cultures are made from exudates from various organs and from the heart blood and studied accordingly

SPECIAL DIRECTIONS

BLOOD CULTURES.

When a blood culture is ordered in the ward, make an appointment with the nurse in charge as to the time of taking it; also, if possible, get some knowledge of the suspected infection. In suspected typhoid infections, make half of the plate cultures with sodium glycocholate agar, and the remainder with plain agar; in suspected mycotic valvular lesions, etc., use dextrose

and plain agars for plating. In all cases inoculate a bottle of plain and one of dextrose broth with the blood.

Preparation of Patient. The nurse prepares the patient's arm for the bacteriologist just preceding the time of taking the blood for culture. Usually the following procedure is carried out: Select the arm which shows the most prominent veins at elbow. Clean the lower third of the patient's upper arm and the upper third of the forearm, with green soap, ether, alcohol, and a 1:1000 solution of bichloride of mercury. Apply the alcohol and bichloride of mercury solution with sterile gauze. Cover the area selected for vein puncture with a piece of sterile gauze moistened with the bichloride of mercury solution, until ready to draw blood for culturing. Do not touch this prepared area with the fingers until the needle is through the skin. If it becomes necessary to palpate the vein, do so through a piece of sterile gauze.

Apparatus and Materials Required. Assemble in tray a sterile 20 c.c. Luer syringe, several sterile syringe needles which must be clean and sharp, six sterile Petri dishes in carrier, ethyl chloride, alcohol lamp and matches, rubber tubing for tourniquet, and clamp. Place six agar tubes with thermometer in large enamel cup, add water and boil until agar is liquified, cool down to 55 C. before leaving for ward. Also include a plain and a dextrose broth flask, and a cup containing 2.5 per cent. cresol solution for disinfection of used syringe and needle.

Procedure. The nurse assists the interne in making the cultures by seeing that a small table covered with a sterile sheet is placed at bedside, arranging plates on table, passing instruments and syringe, and bringing the agar to a temperature of 40 to 42 C. After instruments, etc., are arranged, place tourniquet about upper arm to constrict vein, put plunger in barrel of syringe and securely fasten on the needle, taking care not to infect any of the essential parts. Remove the moistened gauze from the arm, insert needle into vein and draw about 10 c.c. of blood. Release tourniquet after syringe is filled before the needle is withdrawn. Usually the syringe containing the blood is passed to the assistant who adds it as directed to the medium while the interne does the mixing, pouring of plate cultures, etc. Take care not to force the needle off the syringe in ejecting the blood; also work rapidly to make all cultures before the blood coagulates. Add about 1 c.c. of blood to each agar tube, and after adding the blood, mix,

carefully flame the mouth of tube, and pour contents into Petri dish. To pour plates, raise cover of dish at one side just sufficient to admit the end of the tube without touching. Replace cover and spread fluid by slightly tipping and rotating the Petri dish. The remainder of the blood in the syringe is divided between the two broth flasks. After the blood is drawn the puncture in the patient's arm is cleaned, and covered with sterile gauze, which is fixed with adhesive straps.

In the majority of cases blood can be obtained by direct puncture of the vein through the skin. If this fails, a small incision may be made (under ethyl chloride anesthesia) obliquely to the course of the vein, which is then exposed to blunt dissection. This procedure is carried out aseptically, as any minor surgical operation. After the blood is withdrawn from the exposed vein, the incision is closed and surgically dressed, and care is taken to prevent any infection of the patient. After taking cultures, cleanse and disinfect syringe as previously outlined (page 112) and place test tubes in desk boiler for sterilization in autoclave later.

Care of Cultures, Etc. After the agar cultures have hardened, fasten the cover of the dish to the lower portion by wrapping a narrow strip of adhesive tape about the two. Write patient's name, ward, and date on each, and label each flask in the same way. Invert the plates, stack, and place in incubator along with flasks. Make record of case in the bacteriological record book, and also in special blood book where the daily records are entered. If nothing develops in the interval, blood cultures are observed for ten days. Examine cultures daily. If colonies develop on plates make transfers to broth tubes (usually dextrose), and make smears for Gram stain if there is sufficient material. If the broth flasks are turbid make smears for Gram stain, and make transplants to suitable media for type of organism present. If mixed cultures are found, plate material and isolate types. Report findings daily to ward whether positive or negative for first three days, and give final diagnosis as soon as made.

Anaerobic Blood Cultures. Occasionally anaerobic blood cultures are ordered. The procedure is carried out as outlined above, excepting in the preparation of plates and the making of anaerobic agar slant inoculations. Draw blood in the usual way; add about 1 c.c. to either plain or dextrose agar at 40 to 42 C.; and

pour in special deep dishes as used in the Zinsser method of making anaerobic plates. Or, prepare six Petri dish plates as outlined above; later place them in a Novy anaerobic jar; seal; displace air with hydrogen from a generator; then place in incubator for usual period of incubation. Also add about 1 c.c. of blood to each of several plain or dextrose agar slant tubes. The top of the slant should reach only about one-half way to the top of the tube. Spread well over surface, and either place in the Novy anaerobic jar or absorb the oxygen by the alkali-pyrogallic acid method. To carry out the latter simply cut off the cotton plug level with top of the tube, push it down almost to the upper end of the slanted medium, fill and pack the open portion of the tube with dry pyrogallic acid, add a few drops of concentrated solution of sodium hydroxide just sufficient to moisten the powder, then place a fresh cotton plug in the tube, cut top off, force downward, and seal at once with melted paraffin.

To prepare anaerobic plates by the Zinsser method, after the agar medium has hardened, invert the dish, lift out the bottom portion (containing agar culture) and place top downward on a filter paper wet with 1:1000 bichloride of mercury solution. This prevents air contamination. To the top portion of the dish, add pyrogallic acid to the depth of about 2 cm., then add 10 per cent. sodium hydroxide solution to fill dish. At once, partially place the bottom part, open portion downward, in this solution, allowing the contained air to escape in so far as possible. Level, and run liquid paraffin into the space between the walls of the two parts of the dish to seal from air.

If colonies develop, fish, and transplant to agar slants, culture under both aerobic and anaerobic conditions.

COLLECTION OF BLOOD AND SEPARATION OF SERUM FOR WASSERMANN TEST.

Expose patient's arm and place rubber tourniquet about upper portion, taking care not to shut off the pulse in the wrist entirely. Clean arm with green soap, ether, and alcohol, using sterile gauze to apply the alcohol, or disinfect the skin over the vein selected by painting surface with tincture of iodine, which seems to give equally good results. Do not contaminate the prepared area before the blood is withdrawn. Take a large needle (No. 19 gauge 1.5 in. long) which has been sterilized by dry heat

in plugged test tube and place on a dry all-glass syringe. Avoid touching the needle for at least one inch from the point. Insert the needle into the most prominent vein and draw about 10 c.c. of blood. If desired, the skin may be frozen with ethyl chloride before puncturing the vein. After drawing the blood, loosen tourniquet, withdraw needle, and cover the puncture at once with several layers of sterile gauze which is pressed down to stop flow of blood. Discharge blood from the syringe immediately into a sterile test-tube (about 12 cm. x 2.5 cm.); replace plug; place tube in slanting position; and allow blood to clot at room temperature. Clean syringe and needle before blood clots. Remove gauze from patient's arm, clean the arm with alcohol on sterile cotton, touch needle puncture with drop of tincture of iodine on small swab. Cover the area with several layers of sterile gauze, fix in position by means of adhesive strips, and instruct the patient to remove this on the following morning. Place a small (2 cm. x 5 cm.) adhesive strip on blood tube, and label plainly with patient's name, ward, and date. Enter these data in the bacteriological record book, also in the special Wassermann test book and give serial number. After clot is well formed, separate from walls of tube by means of sterile wire, then place in ice box. Remove serum from clot at end of day, place in a 15 c.c. conical centrifuge tube, and throw down all blood cells. See that this tube is properly labelled to prevent confusion or errors. After cells are thrown down, withdraw the clear serum; place in test-tube; label with serial number; stopper with cork; and store in ice box until tests are made. If possible, do not draw blood for Wassermann tests until the day preceeding the day of testing; fresh serum is desirable.

EAR CULTURES.

Make cultures on Loeffler's blood serum medium and dextrose broth. Also make two smears from discharge on clean glass slides. Stain one smear by the Gram method. If Gram-negative bacilli are present, stain the other smear for capsules by the Hiss method. Examine cultures on the following day in the usual way. If Gram-positive, medium sized, palisading bacilli are present, stain with Loeffler's methylene blue and Neisser's stain. To determine species, plate on plain agar, isolate in pure cultures, and prove types.

EYE CULTURES.

Make culture on Loeffler's medium and 5 per cent. blood agar and, if discharge is scant, on dextrose broth. Also make smears on clean slides, if discharge is sufficient, and stain by Gram method. Examine cultures on the following day and make Gram stain. If blood agar culture shows colonies characteristic of the hemoglobinophilic type of organism, use aqueous fuchsin as counterstain in the Gram method. For identification of ordinary types of organism, plate to isolate, fish colonies, and transplant to dextrose broth. Stain by Gram method, and prove types by cultural methods, etc.

Surface seedings on rabbit-blood agar plates are necessary for isolation of the hemoglobinophilic group. Fish fine, dewdrop-like colonies, and transplant to blood agar slant tubes.

THROAT AND NOSE CULTURES.

If diphtheria is suspected, make cultures from membrane on Loeffler blood serum medium, using sterile cotton on wire or wood rod. Also make smears on clean slides, air dry, fix in flame, and stain. Make both Loeffler methylene-blue and Neisser stains on smears and on cultures, then examine for diphtheria bacilli. Allow cultures to incubate from twelve to twenty-four hours, since *B. diphtheriae* seldom develops growth early. To prepare stains from cultures on Loeffler medium with sterile platinum needle remove growth (especially small, gray-white, rather dry-appearing colonies), and emulsify in a drop of sterile salt solution on clean slides. Spread emulsion until rather thin film is formed; air dry; fix in free flame; and stain as directed (p. 107), In doubtful cases, make a Gram stain which will differentiate certain small bacilli from true diphtheria bacilli, and also bring out streptococci more clearly.

Throat and nose cultures are taken from each child before admission to the hospital, and the growth is examined on the following morning. In case there is a visible lesion in the throat at the time the culture is taken, smears should be made on slides and examined for diphtheria bacilli, streptococci, fusiform bacilli, and spirilla. A diagnosis of diphtheria may sometimes be made by direct examination of the exudate, but one should not make a negative report without examining a culture. In Vincent's

angina, smears stained about 30 seconds with aqueous fuchsin usually show the fusiform bacilli and spirilla clearly.

PUS AND EXUDATE CULTURES.

Make smears on clean glass slides, stain one by Gram method, and another by Hiss capsule stain, if indicated by a study of the former. Make cultures on dextrose broth and Loeffler's medium. Centrifuge spinal fluid, clear chest fluid, etc., in a sterile tube and make cultures from the sediment. If either gonococcus or meningococcus is suspected, make cultures on dextrose ascitic agar and dextrose ascitic broth; if *B. influenzae* is suspected, make culture on 5 per cent. blood agar slants. It is often advantageous to incubate the fluid itself. Occasionally a small fibrin clot will separate; in such cases, inoculate medium with this portion. Isolate organisms in pure culture, and prove types.

URINE CULTURES.

Specimens for culture should be taken by catheterization and sent to the laboratory in sterile tubes, utmost care being taken to clean parts before drawing the specimen. In men, after the parts have been well cleaned, the last portion of urine voided may be collected in a sterile tube or directly into sterile broth.

Transfer 1 or 2 c.c. of urine to a dextrose broth tube and to a plain agar slant with a sterile Pasteur pipette. Centrifuge the remainder of the specimen, make smear and stain by Gram method. Examine cultures on the following day.

For identification of species in a growth, plate on plain agar in the usual way, fish different types of colonies, and inoculate into dextrose broth tubes. Make Gram stains of the isolated organisms; and be guided by findings for further cultural tests, etc.

STOOL CULTURES.

To examine stools for typhoid or dysentery bacilli, make a very dilute emulsion in broth. Add a drop of the emulsion to the first of three or four poured plates containing well hardened Endo's medium. By means of a sterile spatula or glass rod with end bent at right angle, spread the emulsion by streaking over each plate consecutively. Or, plate in the usual way on Endo's medium, pouring into large Petri dishes.

After twenty-four to forty-eight hours incubation both the typhoid and the dysentery bacillus appear as small translucent white colonies. Organisms of the colon group form red or pink colonies. Certain other organisms which do not produce red colonies may be present.

Make six to ten fishings from characteristic appearing colonies; inoculate on plain broth tubes; and incubate over night. Make Gram stains, and, if Gram-negative bacilli are found, examine in hanging drop for motility. Inoculate various sugar media for fermentation characteristics; also inoculate Dunham peptone for indol production, and litmus milk for reactions.

VAGINAL SMEARS.

A vaginal smear is made in the examining room from every girl before admission. The smears must be taken and examined immediately. To make smear, separate labia, introduce sterile swab into vagina, and smear by rolling motion on clean slide. Fix by passing three times through flame; stain by the Gram method; and examine. In each case the exact findings should be reported in the bacteriological record book; for example: "Mary X. Vaginal smear: Shows numerous rather degenerated pus cells, many Gram-positive cocci, also small Gram-negative bacilli; no Gram-negative intracellular diplococci found." Never report a case positive for gonococcus, but state that smear shows pus cells containing many Gram-negative intracellular diplococci. As a general rule, a few intracellular Gram-negative diplococci resembling the gonococcus are sufficient to exclude a child, even if not to make a definite diagnosis of gonorrhoea.

Smears that show leucocytes filled with morphologically typical Gram-negative diplococci may be considered positive. Leucocytes showing only an occasional intracellular Gram-negative diplococcus must never be called positive, but should be considered suspicious. If numerous pus cells are present, but no Gram-negative intracellular diplococci are found, the case should be subjected to further examination before giving a report.

EXAMINATION OF SPUTUM.

Examine these as ordered on accompanying tag; open the sputum cups at designated place under hood, and make required smears and cultures. Make stains for tubercle bacilli at same

place; make stains for predominating types of organisms, etc. at work desk; but fix smear on slide in flame before removing from hood. Do not ordinarily take sputum cups to any other part of the laboratory, but if this becomes necessary place them in a basin which can be disinfected. After examination run strong carbolic acid solution in cups, then place them in a pail containing the same solution. Precautions must be taken not to spill sputa, but if this accidentally occurs flood spots at once with 5 per cent. carbolic solution.

For B. influenzae. Make rather thin smears on clean slides, air dry, fix by heat, and stain by Gram method, but counterstain with aqueous fuchsin solution instead of Bismarck brown.

To cultivate the influenza bacillus arrange a series of five or six sterile Petri dishes; almost fill each with sterile physiological salt solution, then select from the sputum one or more of the denser masses by means of a sterile platinum loop, and transfer to the first of the series of dishes. Wash by stirring about with the loop, transfer to the second dish, and continue washing through the series of dishes until the outer portions of the small mass are removed. Place the washed particle on a sterile blood-agar plate, and with a sterile platinum or nichrome seeding spatula make consecutive streaks in parallel lines over the plate, finally continuing this procedure on a second blood-agar plate. If blood-agar plates are not available, the seeding may be carried out on a series of blood-agar tubes by means of a platinum loop. The influenza colony usually appears in about forty-eight hours as a minute, slightly raised, "dew drop" colony. Make fishings of characteristic colonies to blood-agar slants and incubate. If Gram stains made from cultures show the characteristic Gram-negative bacillus, make transplants to both blood-agar and hemoglobin-free-agar slants. If no development takes place within seventy-two hours on the hemoglobin-free-agar, but the characteristic bacillus does appear on the blood-agar controls, report the organism as *B. influenzae.* In making transplants from blood-agar to plain agar in the cultural test, great care must be exercised to prevent carrying over hemoglobin-containing medium. Often a small trace of hemoglobin will be sufficient to permit growth of *B. influenzae* on the plain agar. A transplant from the first to a second plain agar slant may be necessary to determine the diagnosis.

For B. tuberculosis. To make smear of sputum always use new slides which have been well cleaned. With two platinum wires which have been well flamed pick out portions of the sputum. If these are present, select "rice bodies"; avoid clear mucus and pure pus or blood. Make moderately thick smears; label slide with blue pencil; and allow smear to dry in air. Fix by passing slowly three times through the flame. Stain by the Ziehl-Neelsen method (p. 108) and examine for the characteristic red stained bacilli.

Cultures may at times be successfully made by treating the sputum with antiformin (p. 125), and separating and transplanting residue to Lubenau's egg medium. The culturing of the organism is rarely ordered.

For animal inoculation, select masses of sputum or "rice bodies"; wash through series of Petri dishes containing physiological salt solution. Transfer the washed portions to a strong sterile test-tube; add about 1.5 to 2 c.c. sterile salt solution, and emulsify by rubbing with a sterile glass rod. Inject subcutaneously into the inguinal region of a normal guinea-pig, (p. 113).

For Diplococcus pneumoniae. Make two smears on clean glass slides; dry in air and fix in flame. Stain one by the Gram method and the other for capsules by the Hiss capsule stain. Examine for characteristic organisms.

If cultures are ordered for isolation of the organism, wash portions of the sputum as outlined above (under *B. influenzae*), and transfer to either blood-agar slants or Loeffler blood serum medium, and to dextrose broth tube. After growth develops, stain by Gram method, and if characteristic organisms are present, plate from dextrose broth culture with plain agar in the usual way. From growth on plates after twenty-four hours' incubation, fish small bluish-gray colonies and inoculate dextrose broth tubes. If Gram stain shows organisms morphologically resembling the pneumococcus, inoculate one litmus milk and two inulin-serum water tubes each with 3 to 5 drops of the broth culture by means of sterile Pasteur pipette. In the absence of any coagulation of the inoculated media incubate for three days before making final report. Make smears from litmus-milk culture if it shows coagulation, and do capsule stain.

For method of determining group classification of pneumococci, see p 134.

For animal inoculation, wash the sputum in the usual way, prepare emulsion, and inject into mice (p. 135).

EXAMINATION FOR B. TUBERCULOSIS.

Antiformin method for separating B. tuberculosis. By the use of antiformin[1] tubercle bacilli may be freed from other materials and concentrated for examination or inoculation. It is very necessary in the handling of the suspected materials to use glassware which has been thoroughly cleaned, and water for dilution and washing which has been freshly distilled and collected in perfectly clean flasks. Otherwise, certain acid-fast bacteria may enter as contaminants, and give rise to error in diagnosis. Dilute sputum or other fluids to 40 c.c. in a bottle, and mix with 8 c.c antiformin; close with a *clean* rubber stopper, and shake occasionally until the mucus, etc., is dissolved.

In the case of pus, caseous material, minced tissue, etc., add an equal volume of antiformin[2], shake occasionally until the material becomes dissolved, then add several volumes of distilled water. Solution may be hastened by placing the bottle in an incubator at 37 C. for some time. When the materials have gone into solution, pipette into clean tubes, and throw down sediment in centrifuge. Remove supernatant fluid with pipette, and disinfect. Add distilled water; mix; centrifuge again; and repeat the process several times to wash out the antiformin. Finally, from the residue make smear on clean slide; fix by heat; and stain by the Ziehl-Neelsen method. It may be necessary to smear the slide with a very little egg albumen as a fixative before spreading the sediment upon it, but as a rule this is not necessary if the sediment is well washed. If desired, animal inoculations and cultures on Lubenau's egg medium may be made.

A modification of the above method consists of dissolving sputum in the antiformin, then adding ligroin (sp. gr. 0.715 to 0.72) to the solution, and shaking thoroughly. This mixture is then centrifuged, during which process the organisms collect in

1. According to Rosenau, this consists of equal parts of liquor sodæ chlorinatæ, and a 15 per cent. solution of caustic soda. The liquor sodæ chlorinatæ is prepared as follows:

Sodium carbonate	600
Chlorinated lime	400
Distilled water	4000

2. If the antiformin is good, a foaming should take place when it is added to the material and shaken.

a layer between the dissolved sputum and the ligroin. Materials for examination may be removed by means of a capillary pipette, fixed on clean slide, and stained in the usual way. This method is used when only microscopical examinations are required.

All glassware, etc., used in the above procedure must be sterilized, since the tubercle bacilli are not killed by the antiformin.

Spinal fluid. Place the tube containing the fluid, as soon as it is drawn, in the incubator until a delicate clot is formed. Carefully spread out the clot on a clean new slide; fix by heat; and stain in the usual way. If no coagulum appears after four or five hours, scrub finger; prick with needle; and allow a small drop of blood to fall into the tube. This is often found to be efficient.

Stools: Make a watery emulsion of the stool, and centrifuge in clean tubes at moderate speed for ten minutes to remove coarse particles. Remove supernatant fluid to a clean tube and dilute with equal volume of 95 per cent. alcohol; then centrifuge for one half hour or more. From sediment make smears on clean new slides; fix in heat; stain with hot carbol-fuchsin and decolorize in Pappenheim solution. Disinfect the glassware after using.

Urine. Dilute the urine with equal volume of 95 per cent. alcohol, and centrifuge at high speed in sterile clean tubes for one-half hour. Remove supernatant fluid to a clean tube (disinfect); make smears from sediment on clean new slides; stain with carbol-fuchsin in the usual way; and decolorize with Pappenheim's solution. It is well to moisten slide, before making smear, with serum or egg albumen, to fix the bacilli if present.

EXAMINATION FOR TREPONEMA PALLIDUM.

Collection of material. Examinations are usually made of serum from suspected primary lesions, although the treponema may be found in scrapings from secondary lesions. Wash the surface of the ulcer to remove extraneous organisms, then rub with a piece of dry gauze until slightly abraded, but not so that blood exudes to any extent, as this obscures the field. Express serum by squeezing the indurated area, or aspirate with a small Bier cup. If bleeding occurs, wait a few minutes until it stops; then, not infrequently, clear serum may be obtained.

Methods of examination. "Dark Field." Put a drop of the collected serum on a clean thin slide and place over it a cover-

slip, avoiding the inclusion of air bubbles. Put a drop of immersion oil on the under surface of the slide, and place it on the dark field stage also without including air bubbles. Examine with high power dry lens. Illuminate the stage by focusing the light from an arc light on the plain mirror by means of a large condensing lens, and throw out the Abbe condenser.

India ink method (*Burri*). Place a small drop of fresh "Chin Chin" ink upon a clean slide. Collect the serum from the chancre by passing the end of a slide across it; place the serum in the drop of ink; and draw along the slide to make a thin film (ink and serum), as in blood smears. When dry, examine with oil immersion objective for organisms which appear white in a brown or black field.

Wood stain. Make smears by drawing the flat surface of a clean slide across the lesion, or collect a drop of serum on the end of a slide and make a smear similar to a blood smear. Fix preparation 3 minutes in methyl alcohol, then proceed to stain by the same method as that used for blood smears. Place for one minute in eosin solution; drain; cover with methylene azure; and allow to remain for about 3 minutes before washing.

PREPARATION OF BACTERIAL VACCINES.

Culturing Organisms. Before seeding medium, each organism must be separated, and grown in pure culture. Use plain agar slants in tubes or flasks for seeding of such organisms as the staphylococcus group, the *B. coli* and *B. typhosus* groups, etc.; use dextrose broth in flask for the pneumococcus and streptococcus groups, also for the *M. catarrhalis* group; for *B. influenzae* use 5 per cent. blood-agar slants and for the gonococcus use dextrose ascitic-agar slants or starch-agar slants.

Apparatus Required. For the preparation of a vaccine from a bacterial culture, the following articles and materials are necessary: sterile, plugged 1 c.c. pipettes, graduated in 0.01 c.c.; sterile, plugged 5 c.c. bulb pipettes; sterile, plugged 10 c.c. graduated pipettes; sterile plugged Pasteur pipettes 20 cm. long, 2 to 3 c.c. capacity; sterile test-tubes (1.5 x 15 cm.) containing 8 to 10 small beads (2 or 3 mm. diam.); vaccine centrifuge tubes (Hopkins model), which have been plugged with cotton covered with two to three layers of gauze, then placed in metal container, and sterilized by dry heat; filters for bacterial suspension (these are

usually prepared from vaccine centrifuge tubes with broken tips, by loosely packing the bottom with a layer of absorbent cotton 3 to 5 mm., placing the whole, tip downward, in a large test-tube; plugging; and sterilizing by dry heat); clean bottles (10 to 15 c.c.) containing 8 to 10 small beads, which have been plugged with cotton covered with a layer of gauze; then placed in a metal container and sterilized by dry heat; sterile rubber stoppers for bottles.

It is essential to have a water bath with water to the depth of 15 cm. in which a constant temperature can be maintained. The models which are used in chemical laboratories give satisfactory results. In addition, a large metal tripod, a Bunsen burner, and an accurate thermometer are necessary.

Solutions Required: Sterile physiological (0.85 per cent.) salt solution made in the usual way; 0.25 per cent. tricresol, made by adding 0.5 c.c. cresol by means of a sterile 1 c.c. pipette (plugged) to a sterile bottle, and to this 200 c.c. sterile distilled water, sealing the bottle with a sterile rubber stopper, and shaking mixture until cresol is dissolved; 8 per cent. mixture of cresol made in distilled water by placing, with a sterile 1 c.c. pipette, 1 c.c. cresol in a small sterile bottle, and adding 11.5 c.c. sterile distilled water, sealing bottle with sterile rubber stopper, and immediately before withdrawing portions for use vigorously shaking mixture to emulsify it.

Procedure. This is carried out in a clean quiet room, free from dust. After seeding medium, allow to grow at incubator temperature for about 24 hours; then make Gram stains and examine for purity before proceeding. If the culture proves to be pure, in the case of solid medium add a sufficient quantity of sterile salt solution (with a sterile 10 c.c graduated pipette) to make a heavy suspension after washing off the organisms. Place a vaccine centrifuge tube in test-tube stand, remove the plug, and place one of the filters in the mouth of the tube. With a Pasteur pipette* take up the salt solution suspension of organisms and strain into the vaccine centrifuge tube through the cotton in the filter until about 10 c.c. are carried over. In the case of cultures in dextrose broth, remove material from the bottom, and filter

*Always use a small rubber suction bulb on end of the pipette to handle fluids.

into a vaccine centrifuge tube. After transferring the suspension of organisms, place pipette in cylinder of cresol solution on worktable to disinfect. Remove filter, and place in boiler for sterilization. Immediately return sterile plug to vaccine centrifuge tube; bend down free end over edge, and fasten with elastic band; then secure by passing a small strip of adhesive across the top of plug and fastening it down at sides of tube. Label tube with patient's name and type of organism. Counterpoise tube and place in centrifuge. Throw down and pack the bacteria by running machine at the sixth speed for 30 minutes; then with Pasteur pipette remove supernatant fluid (disinfect) and the bacterial sediment to the 0.05 c.c. mark, then add with a sterile 10 c.c. graduated pipette sufficient sterile physiological salt solution to bring volume to 5 c.c. for a 1 per cent. suspension. If less than 0.05 c.c. residue is recovered, add salt solution as necessary to give a 1 per cent. suspension. After adding the salt solution, make a uniform mixture by drawing the fluid and organisms in and out with Pasteur pipette.

Killing Organisms. For this purpose, remove the bacterial suspension from the vaccine centrifuge tube* and place in the sterile test-tube containing the beads. Shake until a perfectly homogeneous suspension results, taking care not to allow the fluid to reach the top of tube or touch plug. When a uniform fine suspension is obtained, remove the plug (place in boiler); sterilize in flame of burner the open end and 4 to 5 cm. below; then take a fresh plug from a sterile test-tube of same size and close. In the meanwhile heat the water in the bath, and bring it to a temperature of 60 C. Submerge the test-tube containing the bacterial suspension, upright in the water bath so that the water level rises within about 2 cm. of the end. Heat the suspension at a constant temperature of 60 C. for one hour.

Sterility Controls on Vaccine. Remove the tube from the water bath after heating is finished, and transfer vaccine to one of the small (10 or 15 c.c.) sterile bottles with a sterile 5 c.c. bulb pipette. Make three control inoculations from vaccine prepared from organisms grown on ordinary medium by adding, with a sterile Pasteur pipette, one drop to an agar slant, one drop to a plain broth tube, and two drops to a flask of plain broth such as is

*When empty, sterilize by boiling.

used for blood cultures. In the case of organisms grown on dextrose broth, dextrose media are used for inoculation of controls, and for organisms growing only upon special media the controls must be made on the same medium. Incubate the inoculated media for three days; if no growth occurs in this period the vaccine may be accepted for use. After making the controls, add tricresol to the vaccine in the same bottle. Vigorously shake the bottle containing the 8 per cent. tricresol mixture until it is emulsified, immediately withdraw 0.25 c.c. by means of a sterile 1 c.c. pipette (plugged) and add to the vaccine (about 5 c.c.) in the small bottle. Securely seal the vaccine bottle with sterile (boiled) rubber stopper; shake thoroughly to put tricresol in solution; then cap stopper and neck with tinfoil, and fasten by passing a strip of adhesive over the top and attaching at sides of bottle. Label the bottle with patient's name, species of organism, volume and concentration (1 per cent.) of suspension, and date; then place in the ice box in a dark place.

Standardization of Vaccine. The standardization is based upon the table of Hopkins for averaged 1 per cent. suspensions prepared as just outlined for the following organisms:

Staphylococcus aureus and albus	=	10	billions	per c.c.
Streptococcus hæmolyticus	=	8	"	" "
Gonococcus	=	8	"	" "
Pneumococcus	=	2.5	"	" "
Bacillus typhosus	=	8	"	" "
Bacillus coli	=	4	"	" "

Dilutions of Vaccine for Use. The dilutions are made from the 1 per cent. stock suspension. To prepare a given dilution, determine the volume of stock solution which contains the desired number of organisms to make the quantity ordered. Measure this amount by means of sterile 1 c.c. plugged pipette; place in small sterile bottle; and add sufficient volume of 0.25 per cent. tricresol solution to bring to required dilution.

Vaccines for intravenous injection are diluted in the same way, except that sterile physiological salt solution must be used as a diluent instead of the tricresol solution. Seal bottle with sterile rubber stopper; cap with tinfoil; and fasten with a strip of adhesive. Label with patient's name, species of organism, number of organisms in each c.c., and date. All vaccines and dilutions should be kept in ice box.

EXAMINATION OF MILK.

Each week the hospital milk (both the certified and the loose) are examined bacteriologically. As a routine, the samples are taken by the bacteriologist early in the morning on test days, and platings are made at once. The plates are allowed seventy-two hours for the development of colonies before the counts are made.

Apparatus and Materials Required. Prepare a number of "water blanks" for dilution purposes as follows: Wash thoroughly a number of square bottles (8 oz.), and dry. Tightly plug each with a stopper made of ordinary cotton covered with cheese cloth, then sterilize with dry heat. To each bottle add 100 c.c. distilled water and mark upper level of fluid with a small adhesive strip, then add 8 c.c. excess to each to compensate for loss by evaporation during sterilization. Cap each with white paper, place in autoclave and heat at 15 lbs. pressure for 30 minutes. Store and use as required, but do not use if volume has been much altered by evaporation as indicated by adhesive strip marker on bottle. Wrap in clean, strong paper a number of Petri dishes in packages of three, and sterilize by dry heat. Plug with cotton a number of 1 c.c. (graduated in 0.01 c.c.) pipettes, place in copper containers and sterilize with dry heat. Prepare in the same way two or three 10 c.c. bulb pipettes. Clean, plug, and cap several large test-tubes (2.5 x 15 cm.); sterilize by dry heat. Sterilize by boiling several rubber stoppers which will fit the mouths of the water-blank bottles. Prepare plain agar tubes for plating and neutral-red lactose peptone fermentation tubes. Cup for liquifying media, and thermometer for regulating the temperature for plating.

Taking of samples. The milk is procured from the steward. When it is possible to mix milk thoroughly without outside contamination, this should be done; otherwise, a sample below the cream zone is taken. Take sterile capped test tubes and container with sterile 10 c.c. bulb pipette to steward's ice-box. After the cover has been removed from the can, place finger over upper end of pipette and plunge pipette quickly downward to a depth of about 25 cm. (10 inches). Draw sample; place in one of the sterile test tubes; plug; and take to the laboratory. Also get a bottle of certified milk with seal intact.

Procedure. After reaching the laboratory, the tests must be

carried out immediately in a quiet room, on table which has been washed free from dust. Shake thoroughly the sample of milk in the test tube; remove 1 c.c. with sterile 1 c.c. pipette; and add to 100 c.c. water blank. Seal with a sterile rubber stopper, and shake vigorously for 3 to 5 minutes. If necessary make a second dilution by removing 10 c.c. of water by means of sterile pipette from water blank, then add 10 c.c. from first dilution, plug with rubber stopper, and shake thoroughly. Arrange two series of Petri dishes on the cleaned table; if the first dilution only is to be plated, put three dishes in each series; if both dilutions are to be used, six dishes to each series. By means of a sterile 1 c.c. pipette take 1 c.c. of the first dilution (1:100), raise cover of first Petri dish, and carefully measure in the amount as near the center as possible; in the same way add 0.5 c.c. to the second dish, and 0.2 c.c. to the third. If the second dilution (1:1000) is used, take another sterile pipette and proceed as with the first. In the meanwhile, liquify plain agar tubes by boiling, and cool to 42 C. To each dish add the contents of agar tube directly to the measured milk dilution, and agitate quickly to insure full mixing and spreading of the medium. Before pouring the medium, remove plug, and in free flame sterilize the lip and 6 to 8 cm. below. The second series of plates is carried out in duplicate as outlined. Label each plate with name of milk, the quantity, the date, and the temperature to be used for incubation. Invert plates, stack each series, and fasten together by a strip of adhesive tape. Place one series in the incubator at 37 C., the other in a dark closet at room temperature, to develop. After seventy-two hours, count the number of colonies which have developed on each. Also inoculate a series of neutral-red lactose peptone fermentation tubes with the milk to test for gas-forming organisms. To the first tube add 1 c.c. undiluted milk; to the second 0.1 c.c. undiluted milk; to the third, 1 c.c. of a dilution of 1:100; and to the fourth, 0.1 c.c. of a dilution of 1:100. Place these tubes in incubator at 37 C. If gas forms, make platings on Endo's medium, fish pink colonies and inoculate on plain broth tubes. Make Gram stains from cultures, and if Gram-negative bacilli are found, place on sugar fermentation tubes to determine types.

In handling the certified milk which is received in original bottles, shake very thoroughly and then remove outer cap. Note

source (dairy farm), date of bottling, and the medical society certifying the milk, all of which data appear on the inner seal. Wipe mouth of bottle and inner cap with clean towel moistened with water, care being taken that towel is sufficiently dry to permit no droplets to remain on bottle or cap. Make dilutions, and proceed in the same way as outlined above.

Counting Colonies. After seventy-two hours incubation, the colonies which have developed are counted on each plate. In general not much difficulty is experienced in determining the number of colonies on a plate made from certified or pasteurized milk. If the colonies are not greatly crowded together, the bottom of the plate may be marked off into fields by drawing lines from the center to the periphery, and counting and recording the number of colonies in each field. Add these totals to get total number on plate and multiply by dilution and volume to get number of colonies per c.c. After making these determinations for each plate, average them and report the average. Time may be saved by using Wolffhügel's colony counter on plates which show numerous colonies evenly distributed. Invert the Petri dish, place the counting plate over it, and count the number of colonies in each of ten of the ruled squares (1 cm.). Take the average for one square, then determine the area of the plate*; and from this calculate the approximate number of colonies on the plate. From this number estimate the number per c.c. of milk, taking into consideration the dilution and the volume plated. Record in bacteriological record book all the data with results of the milk tests.

*Rule: Multiply the square of the radius by 3.1416; the product will be the area required.

SEROLOGICAL TECHNIQUE

DIFFERENTIATION OF TYPES OF PNEUMOCOCCI.

According to Dochez and Gillespie, pneumococci causing acute lobar pneumonia may be classed in four general groups, by means of specific agglutinating sera, certain morphological characteristics, etc. Cultures may be obtained from blood, sputum, etc. Sputum is the material most commonly submitted for examination; therefore, the method of testing this, based upon that originating in the Rockefeller Institute Hospital, will be outlined accordingly.

Apparatus and Materials Required. Sterile Esmarch dishes; sterile small porcelain mortar and pestle; sterile plugged test-tubes (1 x 10 cm.); sterile plugged conical 15 c.c. centrifuge tubes; sterile plugged Pasteur pipettes (18 to 20 cm. long, 2 to 3 c.c. capacity); sterile plugged 1 c.c. pipettes, graduated in 0.01 c.c.; sterile all-glass 2 c.c. syringe with hypodermic needle (2.5 cm. long, 20 gauge); battery jar with cover (for mouse); clean glass slides; metal test-tube stands; water bath (37 C.); sterile instruments (small scissors, scalpels, forceps, etc.) for autopsy; instrument boilers; sterile physiological salt solution; specific agglutinating sera (Groups I and II); sterile ox bile; 95 per cent. alcohol; 5 per cent. carbolic acid solution; culture media (plain broth, blood-agar slants, or plates); white mice for inoculation.

Preparation of Sputum. Collect the sputum in sputum cups, and, if possible, get the portion from the deeper air passages. Make direct smears of the sputum on clean slides; stain by Gram method, also by Hiss capsule stain. If organisms resembling pneumococci are present, arrange and partially fill a series of three or more sterile Esmarch dishes with sterile physiological salt solution. By means of platinum wires fish from the sputum one or more of the denser masses about the size of a bean, and place in the first wash dish. Wash the materials through the series of dishes containing salt solution, handling and manipulating with sterile platinum wires. Transfer the thoroughly washed

masses[1] to the sterile mortar and by means of pestle rub into a homogeneous emulsion, adding sterile salt solution (or bouillon) drop by drop until mixture is sufficiently diluted to pass readily through the syringe needle.

Inoculation of Mouse. Draw 1 or 1.5 c.c. of the emulsified sputum into the sterile syringe through the needle. Select a healthy white mouse for intraperitoneal inoculation. Grasp the animal by the tail with the right hand, then, while gently pulling backwards, grasp the nape of the neck with the thumb and forefinger of the left hand, extend the animal (back to palm of hand) and hold in position for injection by pressing tip of third finger of left hand over root of animal's tail against the base of the thumb. With the freed right hand wipe skin over abdomen with 95 per cent. alcohol on a cotton wipe. Take loaded syringe; puncture the skin in the lower part of abdomen with needle; pass point upward between skin and muscular wall about 2 cm., then puncture the wall and inject the emulsified sputum into the peritoneal cavity. Withdraw needle; place syringe in boiler; wipe skin at point of puncture with 5 per cent. carbolic acid solution on cotton wipe. Place the mouse in the animal jar, which should contain cotton for bedding, also food and water if the animal is not to be killed within a few hours. Label the jar with patient's name, time of injection of animal, and date.

Autopsy of Mouse. In case an early diagnosis is desired, kill the mouse 6 to 8 hours after the injection; otherwise do this later, or await death of the animal. Fasten the dead animal, back downward, to a board by extending legs and placing tacks through the feet. Place in large tray for autopsy. Wash skin over the body with 5 per cent. solution carbolic acid; wipe partially dry with cotton wipe. Lift skin with sterile forceps; make cut just through skin in lower abdominal area; and with sterile scissors extend incision up the median line to the neck region. Dissect back skin on each side with sterile scalpel, exposing the body wall. With sterile forceps lift abdominal wall: make a small incision through wall; and, by means of sterile platinum loop, remove material and make cultures on blood-agar slants and plain broth; then make smears on clean slides. Note the character of the peritoneal exudate; observe especially if it is muci-

1. Cultures may be made also from the washed material if desired.

nous, stringing out on removing platinum loop. Stain the smears by Gram method, and Hiss capsule stain. If numerous characteristic organisms are present, open the abdominal cavity fully; wash out the exudate with 8 to 10 c.c. sterile physiological salt solution; and place it in a sterile 15 c.c. conical centrifuge tube. Wash the cavity by taking about 1 c.c. of the salt solution in a Pasteur pipette with rubber suction nipple, lifting one side of abdominal wall, and discharging the salt solution into the cavity. Draw the fluid back and forth until it becomes quite turbid, then take up and transfer to centrifuge tube. Repeat the process until the cavity and the contained viscera are thoroughly washed. Disinfect the pipettes. After the peritoneal exudate has been removed, open the thorax, under aseptic precautions, and expose the heart. Make an opening to cavity with sterile scissors, then make culture from heart blood on to blood-agar slants and plain broth. Cover body with layer of cotton wet with 5 per cent. carbolic acid, and place, without removing from board, in covered can for cremation. Sterilize all instruments by boiling.

Preparation of Bacterial Suspension. Fasten the plug securely in the mouth of the 15 c.c. centrifuge tube containing the washings from peritoneal cavity of the mouse. Counterpoise and place in the centrifuge, run machine at low speed for 3 to 4 minutes, to throw down leucocytes, etc. Transfer the slightly turbid fluid to a second sterile 15 c.c. conical centrifuge tube; fasten in plug; counterpoise, and place in centrifuge. Throw down the organisms by running machine at sixth speed for 20 to 30 minutes. Remove and disinfect the supernatant fluid, leaving the bacterial residue, to which add sufficient physiological salt solution to give a turbidity about equivalent to a good twenty-four hour broth culture of the pneumococcus.

Agglutinating Serum Test. Place in metal test-tube stand six plugged small sterile test-tubes (1 x 10 cm.). Into each of the first two tubes place 0.3 c.c. of specific agglutinating serum for Group I; into each of the second two, the same quantity of specific agglutinating serum for Group II; in the fifth tube 0.1 c.c. sterile ox bile, and in the last tube, 0.3 c.c. physiological salt solution; finally, add 0.3 c.c. of the homogeneous bacterial suspension; plug tubes; mix by gently shaking; and place in water bath at 37 C. Incubate for two hours, then place in ice-box over night if no definite agglutination has occurred.

Reading and Interpretation of Results. Agglutination when present may be quite variable as to time of appearance and quantity. Occasionally this reaction occurs very quickly, even before the tubes are placed in the bath; other cases vary from first to second hour of incubation even after standing over night in the ice box. The reaction is generally easily recognized by the flocculi of various sizes which settle more or less quickly to bottom of the test-tube. Readings must be checked against the control suspension in sixth tube. The bacterial suspension (pneumococcus) should become clear in the tube containing the ox bile. In case the pneumococcus is overgrown by other organisms in the peritoneal exudate, the test may become impracticable. However, the broth culture made from heart blood of mouse at autopsy may show sufficient growth of the pneumococcus, so that the test can be carried out directly with the culture.

If properly controlled tests show agglutination with either specific agglutinating serum for Group I or Group II, the results are reported accordingly. When no agglutination occurs with either serum, the organism is classed Group IV when the stains made from peritoneal exudate or cultures show that it is not *Pneumococcus mucosus capsulatus.* This organism is classed as Group III. After readings are made from tests, sterilize the test-tubes and contents by boiling 15 minutes in water.

PREPARATION OF SALVARSANIZED SERUM FOR INTRASPINOUS INJECTION.

Preparation of Glassware. With soap and water wash thoroughly large mouthed 8 oz. square bottles, and large mouthed (Rockefeller Institute model) 50 c.c. centrifuge tubes. Rinse in running water; then fill with sulphuric acid-potassium bichromate mixture, and allow to stand over night. Empty cleaning mixture into stock bottle, rinse in running water several times, then wash in stream of flowing water for at least two hours by passing tube to the bottom of each container and allowing the water to overflow at top. After washing rinse three or four times with a liberal amount of distilled water; drain; dry; then stopper mouths with plugs made of cotton wrapped in about four layers of cheese cloth. Trim the free end of the plugs smooth, and cap so as to cover the plug and neck of the container fully. In the case of bottles use strong white paper; for centrifuge tubes,

use clean muslin. Sterilize by dry heat for one hour at 170 to 180 C. After sterilization label each bottle with printed form, and reserve for ward use.

Taking of Blood and Separation of Serum. The blood is drawn from the patient by the ward physician under aseptic precautions, and placed in the specially prepared bottle, which is carefully stoppered. Care should be taken to use a dry syringe and needle in taking the blood, and in emptying the syringe not to apply unnecessary force, nor allow the blood to form foam with air bubbles, which may result in marked hemoglobin staining of the serum. The bottle containing the freshly drawn blood is inclined at a slant, care being taken to prevent the blood from touching the stopper, and coagulation is allowed to take place. After a firm coagulum is formed, the bottle is carried in the same position and placed in special holder in the ice-box in the bacteriological laboratory where it generally remains over night. Usually the serum separates well from the clot, but if it does not, loosen clot from walls of bottle with sterile wire, and allow to stand at room temperature for about one hour, until separation does take place.

Preparation of Serum. When the serum has well separated take bottle to a closed quiet room, where the work-desk has been well washed off with 1:1000 solution of bichloride of mercury. Remove the serum from the clot by means of a clean sterile 25 c.c. pipette which has been well plugged with cotton. The serum may be drawn up by mouth suction or with a rubber bulb attached. Place the serum in one or two of the specially prepared and sterilized 50 c.c. centrifuge tubes. Turn the free margins of the plug over lip of the tube and tie securely with a strong thread or cord. Place the tube in centrifuge and run at third or fourth speed until cells are entirely sedimented, which usually requires about one-half hour. Stop the machine by "stepping down" gradually, finally shutting off the switch after 3 to 5 minutes. Often cells will rise if the machine is stopped too quickly. When the cells are entirely separated from the serum, take the tube to the quiet room, and remove the serum by means of a sterile 10 c.c. pipette, (straight, graduated) with rubber bulb, and measure into a second sterile tube of the same kind. Exercise great care in removing the serum to allow no back flow, or any other manipulation which may cause stirring

up of the sediment at the bottom. In case it is not desired to use the serum full strength value, add sufficient sterile physiological salt solution to give the percentage value of serum ordered. Plug the tube securely, cap with tinfoil, care being taken to keep the lower margin of the foil above the water level when tube is placed in water bath. Finally, place the tube in a water bath, and heat at 56 C. for one hour; the level of the water should never be below that of the serum; if possible it should be kept above. After the serum has been heated at the required temperature for the required time, remove from the bath, put on label with patient's name, ward, percentage value of serum, and date; then place in the ice-box until sent for from ward. In case the serum is deeply stained with hemoglobin, notify the ward physician who has the case in charge before carrying out the final heating, and get instructions whether to proceed or not.

PREPARATION OF NORMAL SERUM FOR SUBCUTANEOUS INJECTION.

The procedure in this case is the same as that outlined under Preparation of Salvarsanized Serum, with the exception that the final step of heating the serum is omitted in this instance, unless ordered by the physician in charge of the case.

BLOOD TESTS FOR TRANSFUSION CASES.

(Hemolysin and Hemagglutinin)

Apparatus Required: Sterile, plugged 1 c.c. pipettes graduated in 0.01 c.c.; sterile 15 c.c. graduated conical centrifuge tubes for separation of serum, washing of corpuscles, etc.; sterile test-tubes (1.5 x 15 cm.) containing about 8 to 10 glass beads (2 or 3 mm. diameter) for defibrinating blood; small glass funnels (3 or 4 cm. diameter); sterile gauze (5 x 5 cm.) to strain defibrinated blood; sterile test-tubes (1.25 x 10 cm.) for serum; sterile test-tubes (2.5 x 10 cm.) for collection of blood for serum; sterile test-tubes (1 x 10 cm.) for test (these tubes must be specially washed in soap and water, rinsed in running tap water; placed in 5 per cent. hydrochloric acid for some time; washed in running tap water; rinsed 2 or 3 times with distilled water; and then dried. The glass should be transparent and show no cloudiness); suitable stands for test-tubes.

Taking of Blood. Draw blood from vein in arm of prospective

donors and of the patient, with a clean, perfectly dry syringe From each donor take about 10 c.c.* of blood, put 1 or 1.5 c.c. in tube containing beads, shake tubes about 15 minutes to defibrinate blood; empty the remainder of the blood into large test-tube; and slant for coagulation and separation of serum. Draw blood from the patient in the same way, controlling the amount in accordance with the number of tests which are to be done. For six donors, defibrinate 1 or 1.5 c.c., and clot about 7 or 8 c.c. for serum. Take history and fill out card for each donor and the patient.

Preparation of Blood. (a) *Serum.* Draw off the serum with Pasteur pipette from the clot as soon as separated, place in 15 c.c. centrifuge tube (labelled with individual's name on strip of adhesive), and throw down cells in the machine. Remove supernatant serum, place in test-tube, and store in ice-box until the test is made.

(b) *Blood cell suspension.* In each case add a few c.c. of physiological salt solution (0.85 per cent.) to the defibrinated blood, and mix. Place 2 or 3 layers of the sterile gauze in a small funnel, through which strain the blood suspension into a 15 c.c. graduated centrifuge tube (label with individual's name); wash blood from defibrinating tube with a second portion of salt solution, and pass through gauze; remove funnel, and add sufficient salt solution to bring total in the tube up to 15 c.c. or more. Throw down the cells in the centrifuge at fourth or fifth speed for 3 to 5 minutes. Remove the supernatant fluid (disinfect), then add the same volume of fresh salt solution, mix by drawing back and forth in Pasteur pipette by means of rubber bulb. Again throw the cells down, and remove the supernatant fluid. Finally, add salt solution again; mix thoroughly; place in centrifuge, and run machine at third speed for 15 minutes to wash and pack cells. Remove supernatant fluid and in case of donors, all corpuscles except 0.2 c.c. (read by graduation marks on tube), then add salt solution to bring volume up to 10 c.c., which on mixing gives a 2 per cent. suspension of corpuscles. Wash patient's blood in the same way, and prepare a 2 per cent. suspension of corpuscles with the salt solution, in such quantity as to cover

*This gives sufficient serum for the tests, including the Wassermann reaction.

fully the required number of tests, based on the number of possible donors.

Procedure. For each donor set up in stand three series of four tubes each, and two extra tubes for blood suspension controls, using the small, specially prepared test-tubes. Mark the tubes in each series as follows:

1st Pc (Patient's cells + Patient's serum).
Ps
2d Pc (Patient's cells + Donor's serum).
Ds
3d Dc (Donor's cells + Patient's serum).
Ps
4th Dc (Donor's cells + Donor's serum).
Ds

Mark the tubes for blood suspension controls either D (donor) or P (patient) as necessary.

The components are combined in each series as indicated above. To the first series add 0.25 c.c. of the respective sera to each tube, likewise the same amounts of 2 per cent. blood suspension; to the second series add 0.1 c.c. serum, 1.5 c.c. salt solution, and 0.25 c.c. blood suspension; to the third series add 0.05 c.c. serum, 2.0 c.c. salt solution, and 0.25 c.c. blood suspension. To the first of the blood suspension control tubes, add 0.5 c.c. of the 0.2 per cent. suspension of corpuscles; to the second tube add 0.25 c.c. of the suspension and 0.25 c.c. of physiological salt solution. In adding the serum, place tip of pipette in bottom of tube and measure in required amount. Always have a uniform suspension of the corpuscles before pipetting to the tubes. After the components are combined, shake tubes thoroughly, and place in bacteriological incubator at 37 C.

Readings. After one hour's incubation examine tubes for reaction. Compare color tint (hemolysis) by holding first and third and second and fourth tubes in each series side by side towards good light. Then slant each tube and observe whether the sedimented cells flow, or not, indicating absence or presence of agglutination. Do not shake tubes; return to incubator for a second incubation of one hour. At the end of this time, make comparative readings for any hemolysis which may be present; then examine the sedimented cells for agglutination by first slant-

ing and then shaking. Note on record cards the results of each reading as made. Place the tubes in the ice-box over night and on the following morning make a reading, which often brings out a delayed agglutination. The following markings are used to indicate the degrees of hemolysis: c (complete); ac (almost complete); p (partial); sl (slight); o (none). Agglutination is indicated by vm (very marked) adherent mass; m (marked) large clumps; rm (rather marked) small clumps; sl (slight) very fine clumps.

RAPID METHOD OF TESTING BLOOD FOR TRANSFUSION CASES.

Rous and Turner have published a method which may be quickly performed and is especially applicable to emergency cases. Their method is based in particular on the hemagglutinin reaction, and the readings are made microscopically.

Apparatus and Materials Required. 1:10 blood mixing pipette (leucocyte type) with capacity of about 0.25 c.c.; capillary pipettes (Wright's); clean slides and cover-slips; small narrow test-tubes; small mixing cups or hollow ground slides; microscope; 10 per cent. sodium citrate solution in distilled water; physiological (0.85 per cent.) salt solution.

Collection of Blood. Rinse blood-mixing pipette with 10 per cent. sodium citrate solution; empty; then draw solution up to mark 1 on the pipette; fill with blood from finger or ear puncture; and expel at once into small test-tube. If flow stops before pipette is filled, expel at once into tube to mix; make new puncture; take up mixture; and draw in required amount of blood. Pressure may be used to cause flow of blood. After taking blood, wash pipette with citrate solution, then with distilled water; rinse with citrate solution; take up the required amount of fresh citrate solution; and proceed to next case.

Mixing Blood Suspension. Make two combinations of the patient's blood with each donor's blood from the citrated blood suspensions; first, 9 parts of patient's blood to 1 part of donor's; second, 1 part of patient's blood to 1 part of donor's. In emergency only the first mixture need be made. Measure by making a mark on capillary tube of pipette, draw blood to that point, then take in air bubble, and blood again, etc., until the required amounts are drawn up. Expel into mixing cup to insure full mixing;

then draw all up into pipette again; seal tip; and hold ready for examination.

Incubation. Keep the pipettes at room temperature, omitting the ordinary higher temperature incubation. It is better to make readings after 15 minutes, but when haste is necessary, they may be made in less time.

Readings. Break the capillary end of each pipette; express a small drop of the blood on to slide; superimpose a large drop of physiological salt solution without mixing; place cover-slip over the drop; and examine the preparation for agglutination under the microscope. In agglutination, the cells are stuck together in masses or irregular heaps, which must be distinguished from the rouleaux of normal blood.

WASSERMANN REACTION.

Among the numerous tests of the clinical laboratory none requires greater technical detail nor demands greater knowledge of the known facts of biochemical reactions than the so-called Wassermann reaction, which is based upon the phenomenon of complement fixation. The reagents entering into this reaction are of biological origin and their chemical compositions are little known. For reliable results, the reagents must be carefully prepared and accurately standardized. Various modifications of the original Wassermann technique have been applied, and, as a result, perhaps no two laboratories perform the test under the same conditions. All attempt, however, to conform with the recognized principles governing the complement fixation phenomenon, and its recognition by means of the hemolytic system as an indicator of the presence or absence of reaction.

The serologist, unlike the analytical chemist, has no definite chemical substance on which the standardization of his reagents may be based, and he must, therefore, look to some one of the several biological reagents available, which is practically constant in value. Serum complement may be used as the primary standard reagent, but the conditions governing its source, preparation, and use must be fixed. By means of complement, the hemolytic immune body (amboceptor of Ehrlich) can be standardized. The immune body is a fairly stable substance, and under certain conditions can be kept for weeks or even months with little or no deterioration in value. After standardization, it serves as a standard reagent for the testing of other samples of complement, a labile substance.

Attention will be directed first to the preparation and standardization of the substances entering the hemolytic system.

SHEEP CORPUSCLES.

Drawing of Blood. Sterilize, by boiling, a bleeding needle, preferably one about 8 to 9 cm. long, with a bore of 2 to 3 mm. Also sterilize, by dry heat, a blood-collecting test-tube measuring

2.5 x 15 cm., containing a small spiral coil of copper wire, and firmly plugged with cotton covered with double layers of cheese cloth, then capped with paper. While the sheep is held by an assistant, trim the wool, using curved scissors, just laterally to the anterior midline of the animal's neck over an area of about 8 x 10 cm.; then lather, and shave clean. Locate the jugular vein by palpation. Wash the shaved area with 5 per cent. solution of carbolic acid. Press the thumb of the left hand over the proximal end of the vessel, causing it to distend with blood; then with the right hand pick up the sterile bleeding needle with a piece of sterile gauze and puncture the vessel.*

Collect the desired amount of blood in the tube as the blood flows from the needle. Plug the tube, and defibrinate the blood by shaking ten to fifteen minutes. After the removal of the needle from the blood vessel, moisten the punctured point with 5 per cent. carbolic acid solution; if the bleeding persists, grasp the skin between the thumb and forefinger over the point, and pinch firmly for about half a minute to form a clot.

It is preferable for a laboratory to keep one or more stock sheep for a supply of blood; if this is not practical, blood may be obtained from an abattoir. In such cases, collect the blood from the severed vessel in sterile tubes; defibrinate, and proceed as outlined. Blood so collected is usually infected, and as a general thing is not safe to use for immunizing purposes. It has been suggested by some workers that the blood be partially sterilized by heating (60 C. for one-half hour), which will destroy at least most of the vegetative forms of organisms, and is said not to injure the antigenic character of the blood cells. Of course, spores are not killed and may be the cause of infection in the animal.

Preparation of Corpuscles. The washing of the defibrinated blood is carried out as follows: Add, with a sterile pipette, 10 to 12 c.c. of the blood to a sterile 50 c.c. centrifuge tube; fill the tube with sterile 0.9 per cent. salt solution; mix thoroughly with pipette, and fasten in plug. Place in the centrifuge and throw down the cells, after which remove the supernatant fluid by

*If desired, the blood may be drawn from the vein in a 20 c.c. all-glass syringe, discharged into the collecting tube, and defibrinated. The drawing of blood in citrated solution is practised by some, but the writer prefers the defibrinated blood.

means of a sterile pipette; add more salt solution and mix well with sedimented cells. Again place in machine and throw down the cells; then remove the supernatant fluid. In all, this procedure is carried out three times. Finally, remove about 5 c.c. of the sedimented cells; place in a 15 c.c. graduated, conical centrifuge tube; add about 10 c.c. of salt solution; mix thoroughly and fasten in plug. Place the tube in the machine and run at high speed for fifteen minutes to pack thoroughly. Remove the supernatant fluid and, if desired, make a reading of the total volume of washed blood cells. The washed, packed corpuscles are now ready for use, either for immunizing or for test purposes.

COMPLEMENT.

Since complement plays the leading role in the Wassermann reaction it becomes necessary to secure a relatively uniform product of high value. Experience has shown that under certain conditions this object may be fairly well accomplished. For this purpose the blood serum of the guinea-pig gives the best results. Serum complement must be used as the primary standard by which the unit value of rabbit immune body is determined. Therefore, in preparing serum for such standardizations, it is necessary to follow rigidly the directions outlined below.

Selection and Care of Animals. Select only normal animals weighing from 350 to 600 gm. Keep in roomy, dry cages or runs in well lighted and freely ventilated rooms. Feed regularly morning and night. Bleed for complement just before the regular feeding time. Keep newly acquired animals, which have been transported, some days before bleeding, to accustom them to their surroundings. Take the animals selected for bleeding from the run; place at once under ether, and bleed without subjecting them to any undue excitement.

Bleeding of Animal. Always place the animal well under the anesthetic by putting a few c.c. of ether on cotton in a small paper cone, or better, in a large-mouthed vaseline bottle which can be slipped over the animal's nose. Take the blood, either by aspirating from the heart, or by bleeding from vessels of the neck. If the heart method is used, shave the chest, and by means of a dry, clean, 10 c.c. all-glass syringe with medium-sized needle (gauge 20) puncture the chest wall close to the sternum through the second or third right interspace. Direct

the needle downward and backward toward the left for 1 to 2 cm. Blood can be easily withdrawn if the needle has entered the heart cavity. Small pigs (350 gm.) will withstand the loss of 4 to 5 c.c. and survive, and larger pigs, of relatively more; or if the animal is to be bled to death, the same size pig may yield 10 to 12 c.c. by this method. If the animal is to be bled to death, trim the hair from the neck of the etherized pig; shave clean, and with a scalpel make an incision about 3 cm. long, through the skin over the trachea. By blunt dissection with forceps expose both carotids; cut through with scissors; allow the blood to flow into a funnel, and collect in a clean, dry, 50 c.c. graduated cylinder. Another method may be used successfully where only a small bleeding is to be made and the animal is to be saved. Prepare the animal for bleeding as outlined above, but make the skin incision parallel to the trachea, and midway between that region and the lateral aspect of the neck. Expose the larger, more superficial vein; cut with scissors; and catch the blood in a clean, dry cylinder as above. When the desired amount is collected, wash the operation wound with 5 per cent. carbolic acid; approximate the edges of the skin and closely suture with catgut which has been well soaked with 5 per cent. carbolic acid solution; wash the closed incision with the carbolic acid solution and place the animal in a cage. Generally the operation wound heals with no difficulty, and several bleedings may be made at intervals of three to four weeks.

Separation of Serum. Allow the blood collected from the animals to stand at room temperature for twenty to thirty minutes until a firm clot is formed. Separate the clot from the wall of the cylinder by a sterile glass rod or wire, then after plugging the cylinder with cotton, place it in the ice box for some hours. Generally the bleedings are made in the late afternoon, just before the night feeding time, and the clotted blood is allowed to stand in the ice-box until the following morning. Then remove the serum from the clot by a pipette; place it in a 15 c.c. centrifuge tube, and throw the cells down. Remove the clear serum and place it in sterile test tubes.

Character of Serum. Occasionally a serum on separation appears quite turbid. Such a serum should be rejected, since it is not suitable for complement. If the serum shows the presence of considerable hemoglobin it should be rejected for the

same reason. A serum suitable for complement should be perfectly transparent and free from hemoglobin, although a slight trace may be permissible under certain conditions.

Complement for Standardizing Immune Body. As pointed out, the hemolytic immune body must be standardized (unit value) primarily by means of complement. To prepare such a complement the serum from five or more guinea-pigs must be pooled. The conditions previously outlined must be rigidly observed. Under such circumstances, different lots of sera so prepared are practically of the same complement value, and may be called "standard complement."

Standardization of Complement. Since it is not practical in most laboratories to bleed five or more guinea-pigs for each series of Wassermann tests, an immune body is standardized and used to standardize the blood-serum from one or more animals. For this purpose, dilute the guinea-pig serum 1:10 with 0.9 per cent. salt solution, and also make a dilution of the hemolytic immune body in 0.9 per cent. salt solution, so that each c.c. contains two units.* Prepare a 5 per cent. suspension of sheep blood cells, in the regular way. Set up a series of test tubes in a suitable stand, and combine the components in the amounts and order given in Table I; then shake thoroughly and place in the water bath at 37 C. for one hour. Make readings at the end of this time and note the smallest amount of the guinea-pig serum which completely destroys all the blood cells. This is the "complement unit." For example, in Table I, which shows an actual test, the unit is 0.08 c.c. Keep the complement dilution, when not in use, in the ice box, instead of at room temperature, as it deteriorates easily. In mixing it do not shake excessively.

HEMOLYTIC IMMUNE BODY (AMBOCEPTOR).

The hemolytic immune body (immune rabbit serum) is most commonly derived from rabbits which have been immunized against the blood cells of sheep.

Immunization of Animal. For immunizing purposes, select normal, healthy rabbits weighing from 1800 to 2500 gm. If

*For example, where the immune body serum has a unit value of $\frac{1}{175}$ c.c. (Table I), $\frac{1}{350}$ c.c. would contain two units; therefore add 0.01 c.c. of this serum to each 8.75 c.c. of 0.9 per cent. salt solution to give the desired value

possible, keep each animal separately caged to prevent fighting and injury. Three animals may be carried through the immunization together. Prepare sheep corpuscles as previously outlined. Pipette off the desired amounts for injection of each animal; place in sterile bottles, and add an equal volume of 0.9 per cent. salt solution to dilute for the injection.

Injection of Animal. Various procedures have been suggested for the immunization of rabbits against sheep blood cells, but experience has shown that none is infallible. Apparently much variation exists between individual animals concerning their ability to form hemolysins. The following procedure has been found, in general, to yield satisfactory results: Fasten a rabbit in the animal holder and expose the marginal ear vein by clipping off the hair and shaving. Pipette 1 c.c. of sterile, packed sheep corpuscles into a small sterile bottle; add an equal volume of sterile 0.9 per cent. salt solution; mix well, and take up in a sterile all-glass syringe through a 3 cm. needle (gauge 21 or 22). Eject any air bubbles present. Grasp the animal's ear firmly with the left hand, and while thus supporting it introduce the needle of the syringe into the vein about 6 to 8 mm. and slowly inject the blood cell suspension. Remove the needle from the vein, and stop the bleeding by compressing that portion of the ear between a fold of sterile absorbent cotton. Repeat this procedure on the second day following, using a dose of 2 c.c. packed corpuscles; then again two days later with a dose of 3 c.c. corpuscles. In each instance use freshly prepared corpuscles and dilute with sterile physiological salt solution.

Sample bleedings may be taken, and the serum tested for hemolytic value, from seven to ten days after the last injection. Usually the antibody content of the blood reaches its maximum concentration at that period. If tests show that the serum is of low titer, the animal may be rejected or more time may be given. If the titer is sufficiently high, collect the blood by placing the animal well under ether and bleeding it to death from the carotid.*

Separation of Serum. Collect about 15 to 18 c.c. of blood aseptically in sterile test-tubes measuring 2.5 x 15 cm.; slant well; and allow to remain at room temperature until firmly clotted. Separate the lower portion of the clot from the side of

* This procedure may be carried out by the method outlined on page 371.

the tube. Place in the ice box over night in an upright position, and the following morning remove the separated serum under aseptic precautions. Place in sterile 15 c.c. centrifuge tubes and throw down free blood cells. Remove the serum and place in well plugged sterile test-tube.

Inactivation of Serum. Place the test-tubes containing the immune serum in water bath at 56 C. for thirty minutes, or longer if the tubes are large. This destroys the complement present, but not the hemolytic immune body (amboceptor).

Standardization of Hemolytic Immune Body. Prepare two dilutions of the inactivated immune serum; the first (1:300) by accurately measuring 0.1 c.c. of the serum by means of a pipette graduated in 1/500 or 1/1000 c.c., adding it to 30 c.c. of 0.9 per cent. salt solution, and thoroughly mixing; the second, (1:3000), by adding 1.0 c.c. of the first dilution to 9.0 c.c. of 0.9 per cent. salt solution. Make a 5 per cent. suspension of sheep blood cells (prepared as for injection purposes) in 0.9 per cent. salt solution. Also prepare fresh standard complement by pooling the serum of at least five guinea-pigs as directed under discussion of complement. Place in a suitable test-tube stand the number of perfectly clean test-tubes (1.2 x 10 cm.) required for the test. Combine the components in the order and amounts indicated in Table II. Shake each tube thoroughly immediately after the blood cell suspension has been added. Place in the water bath at 37 C. for one hour, then remove and note results. Make readings by holding the tube toward the light and observing whether or not a complete destruction of the cells has taken place. If completely hemolyzed, the fluid is clearly transparent, and no residue is left. The smallest amount of the immune body which completely hemolyzes the cells under standard conditions is designated one unit of immune body. Table II shows the results of an actual test on an immune body, which by previous test was found to have a unit value lying somewhere between $\frac{1}{1000}$ and $\frac{1}{2000}$ c.c. As indicated, the unit value was found to be $\frac{1}{1750}$ c.c. Owing to the rather broad limits between the amounts of immune body used in this test, it is well to titrate the same complement back against the standardized immune body, to establish its value in relation to the immune body more accurately in case the same guinea-pig serum is to be used as complement in the Wasser-

mann test at the same time. (For this test, see under Complement).

Preservation and Storage of Immune Body. Sterile immune serum may be preserved for months if placed in sterile ampoules, sealed by flaming the tip, and stored in the dark in a low temperature refrigerator. Usually between 1 and 1.5 c.c. is placed in each ampoule, and the ampoule after sealing is properly labeled, giving titer and date.

ANTIGEN.

It has been found that a number of substances may be used as antigen in the Wassermann test. The original luetic-liver-extract antigen first suggested by Wassermann is seldom used. Perhaps the alcoholic extract of heart (guinea-pig, ox, or human), and the Noguchi acetone-insoluble extract from ox heart are the antigens which are most commonly employed, and in our experience the latter has given very satisfactory results. The selection of any particular antigen depends upon certain requirements, such as a low content of anticomplementary and hemolytic substances, but a high power to bind complement in the presence of serum from luetic patients. Accurate tests must be made on each antigen to measure these factors, and from the results its fitness for practical work is judged.

Preparation of Antigen. The following method, which is a modification of the Noguchi method for the preparation of the acetone-insoluble antigen, has on the whole yielded a satisfactory product. Obtain an ox heart from a freshly slaughtered animal and immediately dissect free from fat, fascia, and tendons. Chop the heart muscle finely by passing twice through a meat chopper. Place the chopped muscle in a meat press, the cylinder of which has been lined with about eight layers of well washed cheese cloth; and apply pressure for a half hour or longer until no more juice can be expressed. Weigh the pressed meat; place it in flasks, and mix with 95 per cent. alcohol in the proportion of 100 gm. to 1000 c.c. Shake the mixture until the lumps are well broken up into small particles. Keep the mixture in a cabinet free from light, at room temperature, for seven to ten days, shaking gently night and morning during that time. Then pour the fluid portion off; collect the residue in washed cheese cloth and express the remaining fluid, filtering the alcoholic solution

through paper. Pour the solution into large shallow dishes to a depth of 1 to 2 cm.; place in a dry room out of the sunlight, and by means of an electric fan evaporate until only a residue remains. Take up the residue in ether so far as soluble. Separate the ether-soluble portion from the insoluble by passing through filter paper. Collect the filtrate in a beaker and allow to evaporate at room temperature until well concentrated. Add the concentrated ether solution to about ten times its volume of chemically pure acetone contained in a tall glass cylinder, while constantly stirring with a glass rod. Allow the resulting precipitate to settle to the bottom of the cylinder; carefully decant the supernatant fluid, and wash the residue two or three times with fresh portions of acetone. Finally wash the residue in a glass evaporating dish, decant the acetone so far as possible, and allow the remainder to evaporate. By means of a spatula, collect the yellow-brown residue, which is the portion used as antigen, into a mass, place in a well fitting, glass-stoppered, wide mouth bottle, and cover with acetone. Stock antigen extract so prepared and stored in the ice box away from the light, will remain unchanged for months.

Testing of Antigen. Besides proving that an antigen possesses strong antigenic properties, it is necessary to prove that it does not contain anticomplementary and hemolytic substances in such amounts as to invalidate its use in practical work. For the purpose of testing an antigen a stock solution of the acetone-insoluble extract is prepared as follows: Place 0.3 gm. of the extract in a strong test-tube and dissolve in 1 to 2 c.c. of ether. To this solution add gradually, while constantly stirring, sufficient chemically pure methyl alcohol to bring the total volume to 10 c.c. The resulting precipitate may be partially dissolved by rubbing out with a glass rod in the solution. Finally remove the residue by filtration. Place the solution in small, dark colored, glass-stoppered bottles and store in the ice box to reserve as stock solution.

Titration of Hemolytic Activity. Make an emulsion of the stock solution, placing in a test tube 1 c.c. of the solution, and to this adding 3 c.c. of 0.9 per cent. salt solution, drop by drop, while constantly shaking. This amount of emulsion is sufficient for both the hemolytic and the anticomplementary titrations which may be carried out at the same time. Arrange in a

suitable stand a series of test tubes like those used in preceding tests, and perform the test as outlined in Table III.

The readings are based on the presence or absence of hemolysis. The maximum amount of the stock solution which shows no hemolysis in the test is taken as its non-hemolytic value, and in the particular instance shown in the table is 0.16 c.c.

Titration of Anticomplementary Power. For this test it is necessary to prepare the following materials: a dilution of hemolytic immune body in 0.9 per cent. salt solution, two units to each c.c.; guinea-pig serum diluted 1:10 in 0.9 per cent. salt solution, then standardized against the immune body dilution to find unit strength; sensitized sheep corpuscles prepared by mixing equal volumes of 5 per cent. corpuscle suspension and the dilution of the immune body (this mixture should stand at room temperature about one hour before using).

Arrange in a stand a series of test-tubes (1.2 x 10 cm.). To these tubes add the emulsion of stock solution, as prepared in the preceding test, in amounts indicated in Table IV (which gives the full procedure to be followed). The maximum amount of the antigen emulsion which shows no inhibition of hemolysis is considered the limit of anticomplementary action. In the table given it is considered as 0.2 c.c.

Titraton of Antigenic Power. If an antigen shows only a small amount of hemolytic and anticomplementary substances, it is tested further to determine its binding power in the presence of luetic sera, and to establish an antigenic unit. This requires at least six or more known positive (++++) luetic sera. In selecting such sera it is necessary to exclude those which in themselves contain anticomplementary substances, also natural hemolytic immune body against sheep corpuscles. This requires preliminary tests upon the positive sera before making tests of the binding power of the antigen. In selecting positive sera for this purpose those showing the presence of bile, fatty substances, hemoglobin, bacterial growth, etc., should be rejected.

To test serum for anticomplementary properties, place a series of test-tubes in a stand, then proceed as outlined in Table V. Dilute the guinea-pig serum 1:10 with salt solution and standardize in the usual way with standard immune body. Also sensitize sheep corpuscles with the same immune body dilution used for standardizing the complement. Those sera (Nos. 3 and 6 in

table) which show inhibition of hemolysis are rejected at once, those passing this test are then tested for the presence of natural immune bodies against sheep corpuscles.

The hemolytic action of the positive luetic sera in the presence of complement is a measure of the amount of natural immune body present. If more than a trace of hemolysis takes place in the tube receiving the larger amount of positive serum, it should be rejected. As in the previous test, standardize guinea-pig serum for complement. Prepare in the regular way a 5 per cent. suspension of sheep corpuscles. Test the positive serum in two amounts as an aid in estimating the amount of natural immune body, if present. Carry out the test as indicated in Table VI. In this case sera No. 5 and No. 6 should be rejected, while serum No. 2 might possibly be used under certain conditions.

For use in the test for the antigenic unit value of the stock solution, pool six or more positive (++++) luetic sera which meet the required conditions. Make emulsions of the stock solution in 0.9 per cent. salt solution by placing 1 c.c. of the stock solution in a test-tube and adding, drop by drop, while constantly shaking, 9 c.c. of the salt solution. From this 1:10 emulsion make another, 1:100, in the same way, and finally from the latter an emulsion of 1:1000. Standardize guinea-pig complement and prepare sensitized sheep corpuscles. Set up a series of test-tubes in a stand as directed in Table VII. Proceed as outlined, using amounts and directions indicated. The least amount of the stock solution (in emulsion) which completely fixes all the complement in the combination is considered one unit. In the case cited the full unit value of the particular antigen falls at tube No. 8, which contains 0.003 c.c. of the original stock solution.

It is well to use if possible ten or more units of antigen in practical work. This type of antigen commonly contains relatively small amounts of hemolytic substances. Therefore, in deciding upon the number of units to be used and in the trial tests, particular attention must be given to the anticomplementary value of the antigen. Never use an amount of the antigen equal to or exceeding half of the anticomplementary amount, as it may cause a false positive reaction. As a general rule, the antigen may be used safely in amounts not exceeding one-third of the anticomplementary dose, provided it contains the required number of units to give complement fixation with positive sera. But

the amount of antigen must not be increased to the extent that marked turbidity of the emulsion obscures the readings of the Wassermann test. Assuming that an antigen whose antigenic unit was found to be 0.003 c.c. was not hemolytic in 0.3 c.c., nor anticomplementary in 0.2 c.c., it could be used safely in twenty units, or 0.06 c.c. If the antigenic value of a stock solution is low, and the anticomplementary value high, these values may approach each other so closely that the antigen is worthless, and must be rejected. All antigens after testing should be run parallel with a good antigen in practical work to prove their value.

Preparation of Patient's Serum, Spinal Fluid, etc. In the preceding paragraphs attention has been given to the preparation and standardization of the biochemical reagents which are used in performing the Wassermann test. It still remains to discuss the preparation of the patient's serum, etc., for test purposes. Collect the blood* in clean, sterile test-tubes; label with patient's name; slant, and allow to remain at room temperature until a firm clot is formed. Loosen the clot from the walls of the tube by means of a strong platinum wire (sterile), and place the tube in the ice box. Each evening remove the sera from the blood samples collected during the day. Remove the serum by means of a pipette; place it in a 15 c.c. conical centrifuge tube; throw down the cells, etc., in the machine. Remove the supernatant fluid; place it in a test-tube; tightly stopper with cork, and then put it in the ice box until time to test. Just previous to testing, inactivate the sera by placing the tubes in the water bath at 55 C. for about thirty minutes.

Draw spinal fluid in the usual way from the patient. If blood cells are present, separate them from the fluid in the centrifuge, and store in ice box until ready for test. Do not inactivate spinal fluid unless evidence of considerable blood is shown, then treat as blood serum. Treat ascitic and other fluids in the same way as spinal fluid.

TECHNIQUE OF REACTION (MODIFIED)

As is evident, the foregoing discussion deals in particular with the materials which must be prepared, and, in several instances, standardized, before the actual Wassermann test can be undertaken. To perform this test the following components must be

* Methods for taking blood are given on page 342.

combined: Patient's serum (spinal fluid, etc.); complement; antigen; sensitized sheep corpuscles, and as diluent, 0.9 per cent. salt solution. Each component must be prepared, standardized, and at hand before starting the test.

In the original Wassermann test the components were combined in such amounts that the final volume was 5 c.c. As a general thing most workers economize materials by doing the test in some aliquot part of the original volume. One-quarter the amount gives satisfactory results, and is used in this laboratory.

It is necessary to have a water bath with a temperature of 37 C. in which the incubation may be carried out. Copper test tube stands with double rows of holes, holding tubes measuring 1.2 x 10 cm. are most suitable for this work.

Make all measurements of materials, in the setting up of the test, with 1 c.c. pipettes divided into $\frac{1}{10}$ c.c. graduations. In measuring the sensitized blood cells use a 5 c.c. pipette graduated in $\frac{1}{10}$ c.c. Set up tubes in the test tube stand by placing two in the front, and one in the back row for each sample of serum or spinal fluid. If serum, add 0.025 c.c. to the first tube in the front row; 0.05 c.c. to the second, and 0.10 c.c. to the back tube. In the case of spinal fluid, add 0.10 c.c. to the first tube, 0.25 c.c. to the second, and 0.5 c.c. to the back tube. Set up controls (positive and negative) in the same way. Since these amounts of serum or spinal fluid are one-quarter the amount used in the full test (5 c.c. volume), use all the other components accordingly. Dilute the guinea-pig serum 1:10 before standardizing, so that the amount of complement which contains one-quarter unit can be used. The amount which just gives complete hemolysis in the complement titration test (one-quarter) is the amount to be used.

Make the antigen emulsion so that 1 c.c. contains the required number of units used for the full test, and use 0.25 c.c. in this case. Prepare the emulsion in the usual way by placing the required amount of the stock solution in a test tube and adding 0.9 per cent. salt solution drop by drop, while constantly shaking, to bring to the required dilution. For example, an antigen has a unit value of 0.003 c.c., and is used twenty units to the c.c. in the full test. In this case each c.c. of the emulsion must contain 0.06 c.c. of the stock solution of antigen. If 30 c.c. of emulsion are required for a given test, then place 1.8 c.c. of the stock solu-

tion in a small flask and add, while constantly shaking, 28.2 c.c. of 0.9 per cent. salt solution. Use 0.25 c.c. to each tube in the one-quarter volume test.

Prepare the sensitized cells as previously mentioned by mixing equal volumes of a hemolytic immune body solution containing two units per c.c. with a 5 per cent. suspension of sheep corpuscles.

In the full test 2 c.c. of this sensitized corpuscle suspension would be used, or 0.5 c.c. in the quarter test. The 0.5 c.c. is equivalent to 0.25 c.c. of 5 per cent. blood suspension and 0.5 unit immune body, which is double the amount some workers use. As a general rule, sheep corpuscles sensitized in this way give sharply defined results. The mixture should be prepared and allowed to stand about one hour before being used in the test.

Add 0.9 per cent. salt solution to bring the total volume in each tube to a constant amount, that is, 0.75 c.c. before the sensitized cells are added, when one-quarter volume test is used.

The order of combining the components varies among different workers. As a routine procedure, pipette the required amount of the serum or spinal fluid into the bottom of the tubes; follow by complement, and salt solution, then shake thoroughly. Next add the antigen, at once shaking thoroughly, and place the tubes in the water bath for incubation. After the first incubation, add the sensitized corpuscles, which must be uniformly mixed. Shake the tubes well and return to the incubator.

Make controls against the antigen by testing it in double the amount used in the test proper to determine if it is anticomplementary in that amount with the system in use. The patient's serum or spinal fluid is omitted in this tube. The hemolytic system is controlled by combining complement, sensitized cells, and diluent to the required volume.

The full procedure with the amounts of components to be used, and the scheme of readings is given in Table VIII.

The reading of the Wassermann reaction is based upon the presence or absence of hemolysis after the test is completed. The degree of hemolysis is inverse in proportion to the degree of complement fixation. The following notation may be used in expressing the different degrees of hemolysis:

C (Complete): Destruction of all blood cells.

AC (Almost complete): Only few blood cells remain.

P (Partial): About one-half blood cells remain.

Sl (Slight): Few blood cells destroyed; supernatant fluid colored slightly red.

O (Negative): No cells destroyed; supernatant fluid water clear.

In marking the strength of the Wassermann reaction, Citron's standard is used as a basis. In order to apply the readings which are used in marking the degree of hemolytic reaction in the test to the Citron standard, the following table has been constructed, and the Wassermann reaction is recorded accordingly:

Hemolysis		Reaction
Tube 1	Tube 2	
O	O	= ++++
Incomplete	O	= +++
Complete (or almost complete)	O	= ++
Complete	Incomplete	= +
Complete	Doubtful	= ±
Complete	Complete	= $\overline{O}$

Key: O: Complete inhibition; no hemolysis.
Sl, P: Incomplete inhibition.
AC: Doubtful inhibition.
C: Complete hemolysis; no inhibition.

In making the readings of the reactions it is well to have the results checked by a second person who is accustomed to the work.

TABLE I.

TITRATION OF COMPLEMENT (ONE-QUARTER UNITS).*

Tube No.	Guinea-pig Serum 1:10 Dilution	Salt Solution 0.9%	Immune Rabbit Serum, 1 c.c.=2 units, e.g., 1-875 Dilution	Sheep Corpuscles 5% Suspension	Water Bath	Hemolysis	Corresponding Amount of Guinea-pig Serum for Whole Units.
	c.c.	c.c.	c.c.	c.c.			c.c.
1	0.3	0.57	0.13	0.25	Incubate 1 hour at 37 C.	Complete	0.12
2	0.25	0.62	0.13	0.25		Complete	0.10
3	0.20	0.67	0.13	0.25		Complete	0.08
4	0.17	0.70	0.13	0.25		Almost Complete	0.068
5	0.13	0.74	0.13	0.25		Almost Complete	0.052
6	0.10	0.77	0.13	0.25		Almost Complete	0.040
7	0.08	0.79	0.13	0.25		Partial	0.032
8	0.06	0.81	0.13	0.25		Slight	0.024
9	—	0.87	0.13	0.25		None	Control

*Modified from Wood, Chemical and Microscopical Diagnosis, 3d ed., 1912.

TABLE II.

TITRATION OF HEMOLYTIC SERUM (ONE-QUARTER UNITS).*

Tube No.	Immune Rabbit Serum	Salt Solution 0.9%	Guinea-pig Serum, 1:10 Dilution	Sheep Corpuscles 5% Suspension	Water Bath	Hemolysis	Corresponding Dilution for Whole Units
	c.c. 1-300	c.c.	c.c.	c.c.			
1	0.75	0.25	0.00	0.25	Incubate 1 hour at 37 C.		Control
2	0.15	0.60	0.25	0.25			1/500
3	0.10	0.65	0.25	0.25			1/750
	1-3000						
4	0.75	0.00	0.25	0.25		Complete	1/1000
5	0.60	0.15	0.25	0.25		Complete	1/1250
6	0.50	0.25	0.25	0.25		Complete	1/1500
7	0.43	0.32	0.25	0.25		Complete	1/1750**
8	0.38	0.37	0.25	0.25		Almost Complete	1/2000**
9	0.33	0.42	0.25	0.25			1/2250**
10	0.30	0.45	0.25	0.25			1/2500
11	0.27	0.48	0.25	0.25			1/2750**
12	0.25	0.50	0.25	0.25			1/3000
13	0.21	0.54	0.25	0.25			1/3500**
14	0.00	0.75	0.25	0.25			Control

*Modified from Wood.

**Approximately correct.

TABLE III.

TITRATION OF HEMOLYTIC ACTIVITY OF ANTIGEN STOCK SOLUTION.* (ONE-QUARTER UNITS).

Tube No.	Antigen Stock Solution	Salt Solution 0.9%	Sheep Corpuscles 5% Suspension	Water Bath	Hemolysis	Amount of Antigen Stock Solution for Whole Unit
	c.c. Emulsion 1-4	c.c.	c.c.			c.c.
1	0.4	0.6	0.25	Incubate 1 hour at 37 C.	Partial	0.4
2	0.3	0.7	0.25		Partial	0.3
3	0.2	0.8	0.25		Slight	0.2
4	0.16	0.84	0.25		None	0.16
5	0.1	0.9	0.25		None	0.1
6	0.06	0.94	0.25		None	0.06
7	0.04	0.96	0.25		None	0.04
8	0.00	1.00	0.25		None	Control

*Modified from Wood.

TABLE IV.

TITRATION OF ANTICOMPLEMENTARY POWER OF ANTIGEN STOCK SOLUTION (ONE-QUARTER UNITS).*

Tube No.	Antigen Stock Solution	Salt Solution 0.9%	Guinea-pig Serum 1-10 Dilution	Water Bath	Sensitized Corpuscles 1 c.c.=1 unit	Water Bath	Hemolysis	Amount of Antigen Stock Solution for Whole Unit.
	c.c. Emulsion 1-4	c.c.	c.c.		c.c.			c.c.
1	0.4	0.1	0.25	Incubate 1 hour at 37 C.	0.5	Incubate 1 hour at 37 C.	None	0.4
2	0.3	0.2	0.25		0.5		Marked	0.3
3	0.2	0.3	0.25		0.5		Complete	0.2
4	0.16	0.34	0.25		0.5		Complete	0.16
5	0.1	0.4	0.25		0.5		Complete	0.1
6	0.06	0.44	0.25		0.5		Complete	0.06
7	0.04	0.46	0.25		0.5		Complete	0.04
8	0.00	0.5	0.25		0.5		Complete	Control

*Modified from Wood.

TABLE V.

TITRATION OF POSITIVE LUETIC SERA FOR ANTICOMPLEMENTARY SUBSTANCES (ONE-QUARTER UNIT).

Specimen	Positive Serum	Guinea-pig Serum 1:10 Dilution	Salt Solution 0.9%	Water Bath	Sensitized Corpuscles 1 c.c.=1 unit	Water Bath	Hemolysis*
	c.c.	c.c.	c.c.		c.c.		
1	0.10	0.2*	0.45	Incubate 1 hour at 37 C.	0.5	Incubate 30 minutes at 37 C.	Complete
2	0.10	0.2	0.45		0.5		Complete
3	0.10	0.2	0.45		0.5		Almost Complete
4	0.10	0.2	0.45		0.5		Complete
5	0.10	0.2	0.45		0.5		Complete
6	0.10	0.2	0.45		0.5		Slight
7	0.10	0.2	0.45		0.5		Complete
8	0.10	0.2	0.45		0.5		Complete
9	0.10	0.2	0.45		0.5		Complete
10	0.10	0.2	0.45		0.5		Complete
11	0.10	0.2	0.45		0.5		Complete
12	0.10	0.2	0.45		0.5		Complete
Control	0.0	0.2	0.55		0.5		Complete

*Variable, depending upon standardization.

TABLE VI

TITRATION OF POSITIVE LUETIC SERA FOR NATURAL IMMUNE BODY AGAINST SHEEP CORPUSCLES (ONE-QUARTER UNITS).

Specimen	Positive Serum	Guinea-pig Serum 1:10 Dilution	Sheep Corpuscles 5% Suspension	Salt Solution 0.9%	Water Bath	Hemolysis
	c.c.	c.c.	c.c.	c.c.		
1	0.025	0.2*	0.25	0.775		O
	0.05	0.2	0.25	0.75		O
2	0.025	0.2	0.25	0.775		O
	0.05	0.2	0.25	0.75		Very Slight
3	0.025	0.2	0.25	0.775		O
	0.05	0.2	0.25	0.75		O
4	0.025	0.2	0.25	0.775		O
	0.05	0.2	0.25	0.75		O
5	0.025	0.2	0.25	0.775		Slight
	0.05	0.2	0.25	0.75		Almost Complete
6	0.025	0.2	0.25	0.775		Almost Complete
	0.05	0.2	0.25	0.75		Complete
7	0.025	0.2	0.25	0.775		O
	0.05	0.2	0.25	0.75		O
8	0.025	0.2	0.25	0.775		O
	0.05	0.2	0.25	0.75		O
9	0.025	0.2	0.25	0.775		O
	0.05	0.2	0.25	0.75		O
10	0.025	0.2	0.25	0.775		O
	0.05	0.2	0.25	0.75		O
Compl. Control No. 1	—	0.2	0.25	0.8		O
Compl. Control No. 2	—	0.4	0.25	0.6		O

Incubate 1 hour at 37 C.

*Variable, depending upon standardization.

TABLE VII.

TITRATION OF ANTIGEN STOCK SOLUTION FOR UNIT VALUE. (ONE-QUARTER UNITS).

Tube No.	Positive Serum (pooled)	Guinea-pig Serum 1:10 Dilution	Antigen Dilutions	Salt Solution 0.9%	Water Bath	Sensitized Corpuscles	Water Bath	Hemolysis	Whole Antigen Unit for Stock Solution
	c.c.	c.c.	c.c.	c.c.		c.c.			c.c.
			1:10 emul.						
1	0.05	0.2*	0.5	0		0.5		None	0.2
2	0.05	0.2	0.25	0.25		0.5		None	0.1
3	0.05	0.2	0.15	0.35		0.5		None	0.06
4	0.05	0.2	0.075	0.425		0.5		None	0.03
5	0.05	0.2	0.05	0.45		0.5		None	0.02
			1:100 emul.						
6	0.05	0.2	0.25	0.25		0.5		None	0.01
7	0.05	0.2	0.15	0.35		0.5		None	0.006
8	0.05	0.2	0.075	0.425		0.5		None	0.003
9	0.05	0.2	0.05	0.45		0.5		Slight	0.002
			1:1000 emul.						
10	0.05	0.2	0.25	0.25		0.5		Partial	0.001
11	0.05	0.2	0.15	0.35		0.5		Almost Complete	0.0006
12	0.05	0.2	0.075	0.425		0.5		Complete	0.0003
13	0.05	0.2	0.05	0.45		0.5		Complete	0.0002
14 Serum Control	0.10	0.2	—	0.45		0.5		Complete	
15 Antigen Control	—	0.2	1:10 emul. 0.5	0.05		0.5		Complete	
16 Hemolytic System	—	0.2	—	0.55		0.5		Complete	

Incubate 1 hour at 37 C.

Incubate 1 hour at 37 C.

*Variable, depending upon standardization.

TABLE VIII. COMPOSITE TABLE SHOWING WASSERMANN TEST WITH READINGS. (ONE-QUARTER UNITS.)

		FRONT ROW									BACK ROW						
Tube No.	Test Substance	Amounts of Test Fluid	Guinea-pig Serum 1-10 Dilution	Salt Solution 0.9%	Antigen Dilution**	Water Bath	Sentitized Corpuscles 1 c.c.=1 unit	Water Bath	Hemolysis	Reaction	Amount of Test Fluid	Guinea-pig Serum 1-10 Dilution	Salt Solution 0.9%	Water Bath	Sensitized Corpuscles 1 c.c.=1 unit	Water Bath	Hemolysis
		c.c.	c.c.	c.c.	c.c.		c.c.				c.c.	c.c.	c.c.		c.c.		
1	Serum, 1	0.025	0.2*	0.275	0.25		0.5		None	Positive	0.10	0.2	0.45		0.5		Complete
2		0.05	0.2	0.25	0.25		0.5		None	(++++)							
3	" 2	0.025	0.2	0.275	0.25		0.5		Partial	Positive	0.10	0.2	0.45		0.5		Complete
4		0.05	0.2	0.25	0.25		0.5		None	(+++)							
5	" 3	0.025	0.2	0.275	0.25		0.5		Almost Complete	Positive	0.10	0.2	0.45		0.5		Complete
6		0.05	0.2	0.25	0.25		0.5		None	(++)							
7	" 4	0.025	0.2	0.275	0.25		0.5		Complete	Positive	0.10	0.2	0.45		0.5		Complete
8		0.05	0.2	0.25	0.25		0.5		Slight	(+)							
9	" 5	0.025	0.2	0.275	0.25		0.5		Complete	Doubtful	0.10	0.2	0.45		0.5		Complete
10		0.05	0.2	0.25	0.25		0.5		Almost Complete	(±)							
11	" 6	0.025	0.2	0.275	0.25		0.5		Complete	Negative	0.10	0.2	0.45		0.5		Complete
12		0.05	0.2	0.25	0.25		0.5		Complete	(o)							
13	" 7	0.025	0.2	0.275	0.25		0.5		Almost Complete	Indefinite	0.10	0.2	0.45		0.5		Almost Complete
14		0.05	0.2	0.25	0.25		0.5		Slight	(Anticomp.)							
15	Spinal fluid, 8	0.10	0.2	0.20	0.25		0.5		Complete	Positive	0.5	0.2	0.05		0.5		Complete
16		0.25	0.2	0.05	0.25		0.5		None	(++)							
17	" " 9	0.10	0.2	0.10	0.25		0.5		Almost Complete	Positive	0.5	0.2	0.05		0.5		Complete
18		0.25	0.2	0.05	0.25		0.5		Partial	(+)							
19	Positive Serum	0.025	0.2	0.275	0.25		0.5		None	Positive	0.10	0.2	0.45		0.5		Complete
20	Control	0.05	0.2	0.25	0.25		0.5		None	(++++)							
21	Negative Serum	0.025	0.2	0.275	0.25		0.5		Complete	Negative	0.10	0.2	0.45		0.5		Complete
22	Control	0.05	0.2	0.25	0.25		0.5		Complete	(o)							
23	Antigen Control	0	0.2	0.05	0.50		0.5		Complete								
24	Hemolytic System	0	0.2	0.55	0		0.5		Complete								

Incubate 1 hour at 37 C. | Incubate 30 minutes at 37 C. | Incubate 1 hour at 37 C. | Incubate 30 minutes at 37 C.

*Variable, depending upon standardization.

**Each c.c. of dilution may contain 10 or more units, but in amount safely below the anticomplementary or hemolytic dose for the system in use.

WIDAL TEST.

Collection of Blood. Usually blood for the Widal test is collected from the finger or ear, although when a culture also is ordered, blood for the two may be drawn at the same time. For the small amount of serum required, prick the finger or ear; touch the curved end of a Wright capsule to the drop; and hold the capsule so that the shaft slants downward at an angie of about 45 degrees. Collect to a depth of about 2 cm. in tube of 5 mm. diameter. Seal in flame, first the curved, then the straight tip of the tube. The capsule may be placed in a carrier, and the clear serum separated from clot in the centrifuge.

Culture of Organism. Each day prepare a fresh culture of *B. typhosus,* either by inoculating a broth tube from fresh agar slant growth and incubating, or by adding physiological salt solution to the agar slant growth and making a thin suspension of the organisms. It is essential that the culture be motile and have no clumps. Do not use cultures which are old; young cultures give best results.

Apparatus Required. Clean cover-slips; hollow ground slides; capillary (Pasteur) pipettes; and a clean porcelain color-mixing tray (such as used in mixing water colors).

Procedure. File and break Wright capsule and set up so that serum may be removed with capillary pipette. Place color-mixing tray in a larger enamel-ware tray, and fill gutter of color tray with physiological salt solution. With vaseline rim margin about well in hollow ground slides. Place clean cover-slips on small strip of blotting paper. Place small rubber nipple on capillary pipette, which should be plugged with cotton. Take up serum in capillary portion of the pipette to a distance of 5 cm. or more, and put one drop in first cup of tray. Empty the pipette and rinse with salt solution. Add nine drops of salt solution to the drop of serum, and mix by drawing in and out of the empty pipette. Put five drops of this mixture into second cup, emptying surplus from the pipette back into the first cup; then rinse pipette in salt solution; and add five drops of salt solution to second cup. Again take five drops from second cup and mix with five drops of salt solution in third. This gives dilutions

1:10, 1:20, and 1:40. Take a portion of the broth culture in the same pipette, and add five drops to the first two cups, and ten to the third, making the dilutions 1:20, 1:40 and 1:80. Make a hanging drop from each dilution by taking a small drop with cool sterile platinum loop and depositing on cover-slip which is placed on a small blotter. Invert slide over coverslip so that the drop comes in center of well, then gently press slide down on cover until the two are firmly sealed by the vaseline ring. Raise, and turn slide over quickly so that the cover-slip is uppermost. Label each slide with patient's name, the dilution, and the time. Make a control of the culture by mixing one loopful with one loopful of the physiological salt on a cover-slip; mount in the same way. This method requires four cover-slips, and the same number of slides. By mounting all the drops on one cover-slip, time as well as slides may be saved, although great care must be exercised not to allow drops to run together, and to avoid confusion in labeling. To proceed, select hollow ground slide with a well of large diameter, ring wtih vaseline. With blue pencil label upper left side from well "control", lower left side "1:40"; upper right side "1:20", lower right "1:80", and add patient's name. Place a cover-slip on small blotter, label blotter with pencil, respective to position of cover-slip, upper left side "1:40", lower left "control", upper right side "1:80", lower right "1:20". With small platinum loop place drops from dilutions and the control in their respective positions, each as near the center as possible without running together. Invert hollow ground slide over the cover-slip so that the corresponding labels fall in the correct positions; seal together by gentle pressure; lift up and turn slide so that the cover-slip is uppermost. After making the hanging drops, keep for one hour at room temperature out of the direct sunlight; then examine with No. 6 or No. 7 lens. Note the amount of clumping and motility. For the sake of uniformity use the following terms:

CLUMPING	*MOTILITY*
None	Unimpaired
Slight	Active
Fair	Fair
Good	Slightly persistent
Almost complete	None
Complete	

The test is worthless if the control shows agglutination at the end of an hour; but a test on non-motile but unagglutinated bacteria gives results of considerable value.

Enter the results in the bacteriological record book, and on the daily record sheet, according to the form given in the following example:

Widal Control: no clumping; activity motile
1:20; good clumping; Slightly persistent motility.
1:40; good clumping; slightly persistent motility.
1:80; good clumping; fair motility.

Test on Dried Blood. To one drop of blood dried on a slide add one drop of physiological salt solution. Rub up with a platinum loop until blood is dissolved as well as possible. Transfer to cup of tray, and add four drops of the salt solution; mix; transfer three drops to second cup; and add three drops of salt solution. Mix; transfer three drops to third cup: and add three drops of salt solution. Add broth culture, two drops to the first cup; three drops to the second cup, six to the third cup. Make hanging drops from each, also make a control; mount as outlined above; and examine.

Always take precautions to prevent spreading of the live typhoid culture; work over large enamel-ware tray containing color tray, etc. Place capillary pipette, used in handling culture, in cylinder on table containing 2.5 per cent. cresol solution; with the small rubber bulb draw into pipette, before submerging it, some of the disinfecting solution. Place the Wright capsule, after finishing, in the boiler for sterilization. Cover color tray as soon as hanging drops are taken for test. When test is finished remove cover and flood the color tray and the bottom of the large work tray containing it with 2.5 per cent. cresol solution, and allow them to stand some hours to disinfect before cleaning. After microscopical readings are made, place the slide and cover intact in boiler for sterilization, or boil in sterilizer at once.

DILUTION OF DIPHTHERIA TOXIN FOR SCHICK REACTION.

The standardized concentrated diphtheria toxin labelled with M. L. D. (minimum lethal dose for guinea-pigs) is procured from the Research Laboratory, Department of Health, New York. It is kept

in ice-box protected from light, and under such conditions retains its strength for a considerable time.

Make a stock dilution from the concentrated toxin by adding sufficient amount of the latter to 0.25 per cent. tricresol solution so that 1 c.c. contains 10 M. L. D. Of this stock solution prepare 10 c.c. each time; place in a sterile amber glass bottle; seal with a sterile rubber stopper; and store in ice-box free from light. Reject the stock solution after two weeks, since owing to deterioration it loses its value.

For the actual test prepare a second dilution by adding 0.1 c.c. of the first or stock solution to 9.9 c.c. sterile physiological salt solution. Place this dilution in a sterile amber glass bottle, seal with sterile rubber stopper, and label "Diphtheria toxin dilution for Schick reaction, 0.2 c.c., contains 1/50 M. L. D.," and add the date of preparation.

The final dilution for the test should not be used later than twenty-four hours after its preparation, and should be kept in the ice-box in dark except while in use. Strict attention should be paid to the original M. L. D. value of each sample of concentrated diphtheria toxin supplied, as this is not constant. Also note carefully the directions which are usually furnished for the preparation of the dilutions.

DILUTION OF LUETIN.

Luetin may be procured already prepared in proper dilution for administration. In such case, when ordered for the ward, after shaking mixture thoroughly, with sterile pipette remove 0.5 to 1 c.c. from the original package, which is kept in the ice-box, and place in a 5 c.c. sterile bottle; seal the bottle with a sterile rubber stopper, then label properly, stating amount to be administered, and the date of preparation. Sometimes luetin (Rockefeller Institute) is supplied as a concentrated emulsion, and must be diluted with sterile physiological salt solution before using. Make the strength of the dilution according to directions furnished. Prepare only small amounts at a time, place in bottle, label, etc., as mentioned above. Always shake bottle thoroughly before removing emulsion to make the dilution. Since the amount to be administered is variable, the directions received with the orginal package must be carefully noted and followed. Keep the dilution in the ice-box when not in use.

BIOCHEMICAL TESTS.

The presence or absence of certain biochemical products formed by bacteria growing under definite conditions is often important in the differential diagnosis of bacterial species. For the recognition of these products, certain chemical reagents are employed. The technique of the most commonly used tests only, with the preparation of the special reagents required, will be considered here.

Acid or Alkali. In the absence of gas production, the question of fermentation of a carbohydrate in a culture is determined by its reaction to an indicator, such as litmus or neutral red. Add a drop of the indicator to the culture and note the color change. Acid gives a light red with litmus, and a straw-yellow with neutral red; while alkali gives a violet-blue with the former, and bright red with the latter.

Quantitative determination of either acid or alkali must be done in measured amounts and titrated with volumetric solution, in the regular way.

To prepare a solution of litmus to be used as an indicator, place 5 gm. of Merck's reagent litmus in a mortar, and rub to a fine powder. Add the powder to 100 c.c. of distilled water in a flask, and heat for one hour in the Arnold sterilizer, shaking occasionally. Filter through paper to remove the insoluble residue, and collect in a clean beaker. Add dilute sulphuric acid drop by drop until the solution turns red. Boil for ten minutes, and if the solution turns blue, add sufficient acid to restore to red color. Add baryta water (saturated solution) drop by drop until the solution becomes a violet color. Allow to stand until residue settles, then filter. Place in flasks, stopper, and sterilize in the Arnold sterilizer for twenty to thirty minutes on each of three consecutive days.

Prepare the neutral red indicator by dissolving 1 gm. of the dye in 100 c.c. of distilled water, and filtering.

Gas Production. At times it is of value to determine the ratio of carbon dioxide to hydrogen in the gas resulting from bacterial

fermentation of a carbohydrate. This may be done roughly by the following method: Carefully mark the lower level of the gas in the closed arm of the fermentation tube, then fill the lower end with a 10 per cent. solution of sodium hydroxide; close with a clean rubber stopper and shake thoroughly. Collect the remaining gas in the closed arm, set upright, and remove the stopper. Take the new level of the gas column; the difference represents the carbon dioxide which has been absorbed by the alkali. The remaining gas is principally hydrogen, which may be demonstrated by the explosion at the open end of the tube by a flame.

Indol. Organisms are grown upon Dunham's peptone solution for three or more days to determine their ability to form indol. At the same time a control tube should be run through. The following test, which is a modification of the Salkowski method, usually gives satisfactory results. Add ten drops of concentrated sulphuric acid to each tube, including the control, and mix well. If a pink color appears, it indicates that both indol and nitrites are present; this is known as the "cholera-red" reaction. If no color appears on standing a short time, add 1 c.c. of freshly prepared 0.01 per cent. solution of sodium nitrite. Allow the solution to flow down the side of the tube and form a layer over the surface of the culture. If indol is present, a pink zone forms at the line of contact between the two solutions.

The Ehrlich test, which is perhaps more accurate, may be made as follows: To about 10 c.c. of the culture add 5 c.c. of a solution composed of paradimethylamidobenzaldehyde, 4 parts; absolute alcohol, 380 parts; concentrated hydrochloric acid, 80 parts. To the mixture of the solution and the culture add 5 c.c. of a saturated watery solution of potassium persulphate; shake thoroughly. Indol is shown by the appearance of a red color.

Nitrites. The reducing power of bacteria is often indicated by the changing of nitrates to nitrites. For this test grow the organism on nitrate broth for four or five days. Mitchell's technique is as follows: Prepare a solution of sulphanilic acid by dissolving 0.5 gm. in 150 c.c. of acetic acid, sp. gr. 1.05 (dilute 400 c.c. of concentrated acetic acid, sp. gr. 1.75, with 700 c.c. of distilled water), and a solution of alpha-naphthlyamin by dissolving 0.1 gm. in 20 c.c. of distilled water: boil; filter, if necessary; then to the filtrate add 180 c.c. of the acetic acid, sp. gr. 1.04.

Keep the solutions separate, but just before making the test mix 2 c.c. of each in a test tube, and add the substance to be tested. Put the mixture in warm water for twenty minutes for reaction, which is indicated by a pink color. Controls should be done on wash water used for tubes, etc.

Vosges-Proskauer Reaction. The organisms are grown for three days in fermentation tubes containing glucose (2 per cent.) peptone solution. To this culture is added 1 c.c. of 50 per cent. potassium hydroxide solution. A positive reaction is indicated by a pink color developing upon prolonged exposure to the air at room temperature. With the true *B. coli* this color does not develop.

ACID-FAST ORGANISMS.

B. tuberculosis
B. smegmatis
B. lepræ
B. Mölleri (grass bacillus)
B. butyricus (butter bacillus of Rabinovitsch)
Some bacteria occurring in distilled water
Some types of Streptothrices

GROUPS OF BACTERIA.

Classified according to Gram Stain.

Positive	Negative
Cocci:	*Cocci:*
M. tetragenus	M. catarrhalis
Pneumococcus group	M. gonorrhœæ
Staphylococcus group	M. intracellularis meningitidis and related types
Streptococcus group	M. melitensis
	M. pharyngis siccus
	Micrococci of chromogenic group
Bacilli:	*Bacilli:*
B. aërogenes capsulatus	B. acidi lactici
B. anthracis	B. cloacæ
B. botulinus	B. coli group
B. diphtheriæ group	B. dysenteriæ group
B. tetani	B. enteritidis
B. tuberculosis and other acid-fast bacilli	B. fæcalis alkaligenes
	B. influenzæ group
	B. Koch-Weeks
	B. lactis aërogenes
	B. maligni œdematis
	B. mallei
	B. Morax-Axenfeld
	B. mucosus capsulatus
	B. pertussis group
	B. pestis
	B. proteus
	B. pyocyaneus
	B. typhosus group
	Spirillum:
	S. choleræ

FERMENTATION REACTIONS OF GRAM-NEGATIVE GROUP OF COCCI ON SUGAR SERUM-WATERS*

Strains	Dextrose	Maltose	Levulose	Saccharose	Lactose	Galactose
Meningococcus	+	+	O	O	O	O
Pseudomeningococcus	+	+	O	O	O	O
Gonococcus	+	O	O	O	O	O
Micrococcus catarrhalis	O	O	O	O	O	O
Micrococcus pharyngis siccus	+	+	+	+	O	O
Chromogenic group I	+	+	+	+	O	O
Chromogenic group II	+	+	+	O	O	O
Chromogenic group III	+	+	O	O	O	O

*Abridged from Elser and Huntoon.
+ = fermentation
O = no fermentation.

TABLE FOR DILUTION OF "OLD TUBERCULIN" OF KOCH
(After Park and Williams)

Dilutions	Amount of Tuberculin	Amt. of diluent*	Content of tuberculin terms of finished product
A	1 c.c.	9 c.c.	1 c.c. = 0.1 c.c. or gm., or 100 c.mm. or mgm.
B	1 c.c. dilution A	9 c.c.	1 c.c. = 0.01 c.c. or gm., or 10 c.mm. or mgm.
C	1 c.c. dilution B	9 c.c.	1 c.c. = 0.001 c.c. or gm., or 1 c.mm. or mgm.
D	1 c.c. dilution C	9 c.c.	1 c.c. = 0.0001 c.c. or gm., or 0.1 c.mm. or mgm.
E	1 c.c. dilution D	9 c.c.	1 c.c. = 0.00001 c.c. or gm., or 0.01 c.mm. or mgm.
F	1 c.c. dilution E	9 c.c.	1 c.c. = 0.000001 c.c. or gm., or 0.001 c.mm. or mgm.

*The dilutions should be made with sterile physiological salt solution containing about 0.25 per cent. tricresol.

FERMENTATION REACTIONS OF B. DIPHTHERIÆ GROUP*
HISS MEDIUM (TEN DAYS' GROWTH)

ORGANISM	Glucose	Lactose	Saccharose	Galactose	Maltose	Levulose	Mannit.	Dextrin	Glycerin
B. diphtheriæ, virulent and avirulent	C A	C A	O	C A	C A	C A	O	C A	C A
Hoffmann bacillus..	O	O	O	O	O	O	O	O	O
Xerosis bacillus...	C A	O	O	O	O	C A	O	O	C A
B. coryzæ.....	C A	O	O	C A	O	C A	O	O	O

C = coagulation
A = acid
O = no reaction
*Abridged from Graham-Smith.

FERMENTATION REACTIONS‡ OF A GENERAL GROUP OF GRAM-NEGATIVE BACILLI

Organism	Dextrin	Dextrose	Galactose	Lactose	Levulose	Maltose	Mannit	Saccharose	Litmus Milk	Gelatine Liquefaction	Indol	Motility
B. acidi lactici	++	++	++	++	++	++	++	—	+ c*	—	+*	—
B. cloacæ	++	++	++	++	++	++	++	++	c p	+	—	+
B. coli communis	++	++	++	++	++	++	++	—	c	—	+	+
B. coli communior	++	++	++	++	++	++	++	++	c	—	+	+
B. dysentery (Shiga)	—	+	—	—	—	—	—	—	+ —	—	—	—
B. dysentery (Flexner)	+	+	+	—	+	+	+	+	+ —	—	+	—
B. dysentery (Hiss-Russell)	—	+	+	—	+	—	+	—	+ —	—	+	—
B. dysentery (Rosen)	+	+	+	+	+	+	+	—	+	—	+	+
B. enteritidis	++	++	++	—	++	++	++	—	—	—	—	+
B. fæcalis alkaligenes	—	—	—	—	—	—	—	—	—	—	—	+
B. lactis aérogenes	++	++	++	++	++	++	++	++	c	—	+	—
B. mucosus capsulatus	++	++	++	++	++	++	++	++	c	—	+	—
B. Morgan No. 1	?	++	?	—	?	?	—	—	+ —	—	?	+
B. paracolon (Day)	++	++	++	—	++	++	++	—	+ —	—	?	+
B. paratyphosus, alpha	—	++	++	—	++	++	++	—	+	—	—	+
B. paratyphosus, beta	—	++	++	—	++	++	++	—	+ —	—	—	+
B. proteus	?	++	++	—	++	++	—	++	p	+	+	+
B. pyogenes fœtidus	?	+	+	+	+	+	+	+	+	—	?	+
B. typhosus	+	+	+	—	+	+	+	—	+ —	—	—	+

‡Exceptions in some details may be encountered among certain strains of these organisms.

*Usually

Carbohydrate media

— = no fermentation
+ = acid formation
++ = acid and gas formation

Nomenclature

Milk

— = no fermentation, alkaline reaction
+ = acidity with terminal alkalinity
+ = acid
c = coagulation
p = peptonization

SERIAL

P
Med
S
v.4

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